

**ELECTROCARDIOGRAMS SUMMARY  
WEEK 1  
MALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 35 MG/KG	GROUP 4 120, 80 MG/KG
<b>HEART RATE (bpm)</b>					
-----					
Before dosing	MEAN	118	98	118	122
	ST.DEV.	20	10	17	28
	N	6	4	4	6
1h after dosing	MEAN	105	103	118	112
	ST.DEV.	22	13	26	33
	N	6	4	4	6
<b>P AMPLITUDE (mV)</b>					
-----					
Before dosing	MEAN	0.23	0.18	0.21	0.27
	ST.DEV.	0.05	0.09	0.11	0.06
	N	6	4	4	6
1h after dosing	MEAN	0.19	0.20	0.28	0.28
	ST.DEV.	0.06	0.08	0.12	0.08
	N	6	4	4	6
<b>P DURATION (ms)</b>					
-----					
Before dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	6
1h after dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	6
<b>P-Q INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	32	34	38	35
	ST.DEV.	7	14	15	14
	N	6	4	4	6
1h after dosing	MEAN	37	39	38	39
	ST.DEV.	8	13	15	12
	N	6	4	4	6
<b>QRS INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	6
1h after dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	6
<b>Q-T INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	190	193	198	185
	ST.DEV.	9	10	5	15
	N	6	4	4	6
1h after dosing	MEAN	198	195	199	218
	ST.DEV.	12	10	6	31
	N	6	4	4	6

\*\*\*: Dunnett-test based on pooled variance sig. at 5% or 1% level.  
\* : t-test significant at 5% or 1% level; before vs. after dosing.

**ELECTROCARDIOGRAMS SUMMARY  
WEEK 1  
FEMALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 35 MG/KG	GROUP 4 120, 80 MG/KG
<b>HEART RATE (bpm)</b>					
-----					
Before dosing	MEAN	138	130	105	117
	ST.DEV.	41	18	25	25
	N	6	4	4	6
1h after dosing	MEAN	123	123	108	108
	ST.DEV.	38	13	30	19
	N	6	4	4	6
<b>P AMPLITUDE (mV)</b>					
-----					
Before dosing	MEAN	0.29	0.25	0.21	0.23
	ST.DEV.	0.09	0.06	0.06	0.07
	N	6	4	4	6
1h after dosing	MEAN	0.29	0.21	0.21	0.25
	ST.DEV.	0.11	0.03	0.05	0.09
	N	6	4	4	6
<b>P DURATION (ms)</b>					
-----					
Before dosing	MEAN	43	40	40	42
	ST.DEV.	5	0	0	4
	N	6	4	4	6
1h after dosing	MEAN	42	40	40	43
	ST.DEV.	4	0	0	3
	N	6	4	4	6
<b>P-Q INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	86	85	88	94
	ST.DEV.	8	6	10	12
	N	6	4	4	6
1h after dosing	MEAN	89	86	89	104
	ST.DEV.	9	5	11	13
	N	6	4	4	6
<b>QRS INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	6
1h after dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	6
<b>Q-T INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	187	184	203	192
	ST.DEV.	16	5	5	15
	N	6	4	4	6
1h after dosing	MEAN	184	195	210	237 ***
	ST.DEV.	18	10	18	15
	N	6	4	4	6

\*\*\*: Dunnett-test based on pooled variance sig. at 5% or 1% level.  
 \*\*B. test significant at 5% or 1% level; before vs. after dosing.

**ELECTROCARDIOGRAMS SUMMARY  
WEEK 13  
MALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 15 MG/KG	GROUP 4 120, 80 MG/K
<b>HEART RATE (bpm)</b>					
-----					
Before dosing	MEAN	95	88	110	133
	ST.DEV.	14	17	26	46
	N	6	4	4	4
1h after dosing	MEAN	95	100	105	110
	ST.DEV.	10	14	24	18
	N	6	4	4	4
<b>P AMPLITUDE (mV)</b>					
-----					
Before dosing	MEAN	0.18	0.19	0.23	0.31
	ST.DEV.	0.05	0.06	0.10	0.09
	N	6	4	4	4
1h after dosing	MEAN	0.18	0.19	0.25	0.25
	ST.DEV.	0.05	0.06	0.15	0.07
	N	6	4	4	4
<b>P DURATION (ms)</b>					
-----					
Before dosing	MEAN	40	40	43	43
	ST.DEV.	0	0	5	5
	N	6	4	4	4
1h after dosing	MEAN	40	40	43	45
	ST.DEV.	0	0	5	6
	N	6	4	4	4
<b>P-Q INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	87	84	90	78
	ST.DEV.	10	13	15	5
	N	6	4	4	4
1h after dosing	MEAN	85	83	91	83
	ST.DEV.	9	12	15	13
	N	6	4	4	4
<b>QRS INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	4
1h after dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	4
<b>Q-T INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	207	208	203	195 *
	ST.DEV.	11	15	13	10
	N	6	4	4	4
1h after dosing	MEAN	205	205	213	210 *
	ST.DEV.	10	10	15	12
	N	6	4	4	4

\*/\*: Dunnett-test based on pooled variance sig. at 5% or 1% level.  
\* : t-test significant at 5% or 1% level; before vs. after dosing.

**ELECTROCARDIOGRAMS SUMMARY**  
**WEEK 13**  
**FEMALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 35 MG/KG	GROUP 4 120, 80 MG/KG
<b>HEART RATE (bpm)</b>					
-----					
Before dosing	MEAN	130	135	105	108
	ST.DEV.	38	26	39	29
	N	6	4	4	6
1h after dosing	MEAN	123	133	93	105
	ST.DEV.	27	22	21	19
	N	6	4	4	6
<b>P AMPLITUDE (mV)</b>					
-----					
Before dosing	MEAN	0.27	0.29	0.25	0.22
	ST.DEV.	0.08	0.13	0.04	0.04
	N	6	4	4	6
1h after dosing	MEAN	0.26	0.28	0.23	0.23
	ST.DEV.	0.07	0.09	0.03	0.04
	N	6	4	4	6
<b>P DURATION (ms)</b>					
-----					
Before dosing	MEAN	43	40	43	43
	ST.DEV.	5	0	5	5
	N	6	4	4	6
1h after dosing	MEAN	43	40	43	43
	ST.DEV.	5	0	5	5
	N	6	4	4	6
<b>P-Q INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	88	90	94	95
	ST.DEV.	9	8	11	12
	N	6	4	4	6
1h after dosing	MEAN	88	95	98	94
	ST.DEV.	9	6	13	13
	N	6	4	4	6
<b>QRS INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	6
1h after dosing	MEAN	40	40	40	40
	ST.DEV.	0	0	0	0
	N	6	4	4	6
<b>Q-T INTERVAL (ms)</b>					
-----					
Before dosing	MEAN	183	186	195	200
	ST.DEV.	15	8	6	18
	N	6	4	4	6
1h after dosing	MEAN	188	193	220	212
	ST.DEV.	13	26	14	18
	N	6	4	4	6

\*\*\*: Dunnett-test based on pooled variance sig. at 5% or 1% level.  
 \*\*\*: test significant at 5- or 1% level: before vs. after dosing.

**ELECTROCARDIOGRAMS (Q-T CORRECTED) SUMMARY  
PRETEST  
MALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 35 MG/KG	GROUP 4 120, 80 MG/KG
-----					
Van de Water's correction					
-----					
Pretest	MEAN	227	223	230	223
	ST.DEV.	15	7	8	8
	N	6	4	4	6
-----					
Fridericia's correction					
-----					
Pretest	MEAN	231	223	233	228
	ST.DEV.	17	8	11	9
	N	6	4	4	6

**ELECTROCARDIOGRAMS (Q-T CORRECTED) SUMMARY  
PRETEST  
FEMALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 35 MG/KG	GROUP 4 120, 80 MG/KG
-----					
Van de Water's correction					
-----					
Pretest	MEAN	225	228	230	227
	ST.DEV.	16	9	1	4
	N	6	4	4	6
-----					
Fridericia's correction					
-----					
Pretest	MEAN	231	235	232	231
	ST.DEV.	20	14	5	7
	N	6	4	4	6

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**ELECTROCARDIOGRAMS (Q-T CORRECTED) SUMMARY  
WEEK 1  
MALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 15 MG/KG	GROUP 4 120, 80 MG/KG
<b>Van de Water's correction</b>					
Before dosing	MEAN	232	226	240	227
	ST.DEV.	8	8	4	12
	N	6	4	4	6
1h after dosing	MEAN	234	231	240	254 *
	ST.DEV.	10	5	9	19
	N	6	4	4	6
<b>Fridericia's correction</b>					
Before dosing	MEAN	237	226	247	233
	ST.DEV.	12	9	7	18
	N	6	4	4	6
1h after dosing	MEAN	238	233	247	264 *
	ST.DEV.	13	5	15	22
	N	6	4	4	6

**ELECTROCARDIOGRAMS (Q-T CORRECTED) SUMMARY  
WEEK 1  
FEMALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 35 MG/KG	GROUP 4 120, 80 MG/KG
<b>Van de Water's correction</b>					
Before dosing	MEAN	233	230	238	232
	ST.DEV.	13	8	8	11
	N	6	4	4	6
1h after dosing	MEAN	225	240	246 *	275 **
	ST.DEV.	11	13	13	9
	N	6	4	4	6
<b>Fridericia's correction</b>					
Before dosing	MEAN	243	238	243	230
	ST.DEV.	20	15	15	16
	N	6	4	4	6
1h after dosing	MEAN	230	247	253	287 **
	ST.DEV.	13	19	16	9
	N	6	4	4	6

**ELECTROCARDIOGRAMS (Q-T CORRECTED) SUMMARY  
WEEK 13  
MALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 35 MG/KG	GROUP 4 120, 80 MG/KG
<b>Van de Water's correction</b>					
Before dosing	MEAN	238	233	240	230
	ST.DEV.	10	6	4	15
	N	6	4	4	4
1h after dosing	MEAN	237	239	248	249
	ST.DEV.	8	3	12	5
	N	6	4	4	4
<b>Fridericia's correction</b>					
Before dosing	MEAN	240	234	246	239
	ST.DEV.	12	5	8	26
	N	6	4	4	4
1h after dosing	MEAN	239	242	254	256 *
	ST.DEV.	10	4	16	5
	N	6	4	4	4

\*\*\*: Dunnett-test based on pooled variance sig. at 5% or 1% level.  
\*: t-test significant at 5% or 1% level; before vs. after dosing.

**ELECTROCARDIOGRAMS (Q-T CORRECTED) SUMMARY  
WEEK 13  
FEMALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 35 MG/KG	GROUP 4 120, 80 MG/KG
<b>Van de Water's correction</b>					
Before dosing	MEAN	227	234	228	236
	ST.DEV.	8	3	13	11
	N	6	4	4	6
1h after dosing	MEAN	231	239	249	248
	ST.DEV.	10	18	14	13
	N	6	4	4	6
<b>Fridericia's correction</b>					
Before dosing	MEAN	234	243	232	241
	ST.DEV.	11	8	22	14
	N	6	4	4	6
1h after dosing	MEAN	238	249	253	254
	ST.DEV.	14	21	19	14
	N	6	4	4	6

\*\*\*: Dunnett-test based on pooled variance sig. at 5% or 1% level.  
\*: t-test significant at 5% or 1% level; before vs. after dosing.

**ELECTROCARDIOGRAMS (Q-T CORRECTED) SUMMARY  
AFTER RECOVERY  
MALES**

		GROUP 1 0 MG/KG	GROUP 4 120, 80 MG/KG
Van de Water's correction			
Recovery	MEAN	229	244
	ST. DEV.	0	---
	N	2	1
Fridericia's correction			
Recovery	MEAN	229	252
	ST. DEV.	0	---
	N	2	1

**ELECTROCARDIOGRAMS (Q-T CORRECTED) SUMMARY  
AFTER RECOVERY  
FEMALES**

		GROUP 1 0 MG/KG	GROUP 4 120, 80 MG/KG
Van de Water's correction			
Recovery	MEAN	244	241
	ST. DEV.	12	13
	N	2	2
Fridericia's correction			
Recovery	MEAN	254	255
	ST. DEV.	24	15
	N	2	2

**Hematology:** Slight ↑red blood cell count, hematocrit and hemoglobin in HD M (120/80 mg/kg/day) compared to controls (not significant, as the pre-test values in the HD animals were higher than in control pre-test values) in Weeks 6 and 13, higher in Week 13; no treatment-related findings after 4-week recovery.

**Clinical chemistry:** Slight ↑sodium compared to baseline in HDM and HDF in Weeks 6 and 13, with minimal increases at the MD in Week 13; no associated changes in electrolytes, no treatment-related findings after the 4-week recovery period.

**Urinalysis:** No treatment-related effects.

**Gross pathology:** No treatment-related effects. 2 HD M that died or were sacrificed during the study (Nos 18 and 20) showed dark red thymus and jejunum discoloration, associated with acute congestion/hemorrhages commonly found in animals found dead or incompletely exsanguinated.

**Organ weights:** No treatment-related effects, as all differences from controls were within range of normal biologic variation.

**Histopathology:**

- **↑Incidence and severity of thymic atrophy**
  - Several M given 35 (2/4) and 120/80 (3/4) mg/kg/day, vs. 2/4 in the controls and 1/4 in the LD male dogs
  - Mean severity 1.0, 1.0, 3.0, and 2.3 in the control, LD, MD, and HD dogs, respectively (scale of 0-4 with increasing severity)
  - Possibly associated to treatment-induced stress response
- **↑Incidence of chronic inflammation of the prostate**
  - Several M given 35 (4/4) and 120/80 (4/4) mg/kg/day, compared to finding in 2/4 controls and 1/4 LD M
  - No treatment-related effects on severity: mean severity 2.5, 1.0, 2.0, and 2.3 in the control, LD, MD, and HD dogs, respectively (scale 0-4)
  - Probably not a direct treatment effect, but due to poor general health condition, also common in sexually mature M dogs
- **↑Incidence and severity of adrenal corticocellular hypertrophy**
  - M given 35 (2/4) and 120/80 (4/4) mg/kg/day, vs. 1/4 in the controls
  - No treatment-related effects in the MD and HD F
  - within normal range of biological variation
  - without correlation to adrenal weight in the affected animals
- No histopathology findings in the 4-week recovery animals

The notable histopathology findings are presented in the following table:

Daily Dose (mg/kg)	0 (Control)		10		35		120/80	
	♂M	♀F	♂M	♀F	♂M	♀F	♂M	♀F
Thymus - atrophy	2	1	1	0	2	2	3	0
Prostate gland - chronic inflammation	2	n.d.	1	n.d.	4	n.d.	4	n.d.

n.d. = not determined.

**Toxicokinetics:** Test article exposure was demonstrated in all treated dogs, with high intra-individual variability in plasma concentrations, particularly at the HD. There was a greater than dose-proportional increase in plasma concentrations. Peak plasma tapentadol concentrations observed at approximately 0.5-1 h after dosing. The results of the toxicokinetic evaluation are presented in the following table (provided from the original NDA submission):

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ON ORIGINAL**

**Table A: Mean C<sub>max</sub> and AUC<sub>0-t</sub> values of CG5503 base**

Dose CG5503 [mg/kg]	Time [h]	C <sub>max</sub> ± S.D. [ng/ml]		AUC <sub>0-t</sub> ± S.D. [h-ng/ml]	
		male	female	male	female
10	day 1	13.7 ± 12.6	10.1 ± 3.22	30.9 ± 16.7	17.5 ± 3.85
	day 91	4.19 ± 1.19	4.42 ± 1.34	18.9 ± 3.31	17.1 ± 2.98
35	day 1	26.9 ± 13.6	43.4 ± 11.0	64.9 ± 22.9	86.6 ± 7.25
	day 91	37.1 ± 19.1	40.5 ± 11.1	101 ± 14.2	110 ± 42.3
120/80 <sup>1)</sup>	day 1	701 ± 1192	245 ± 205	846 ± 1089	513 ± 253
	day 91	316 ± 382	338 ± 534	491 ± 447	511 ± 469

1) The dose had to be reduced from 120 mg/kg to 80 mg/kg starting with day 22

**Other:** Evaluation of hepatic microsomal enzymes showed significant induction of aminopyrine N-demethylase activity in M and F. Also, glucuronyltransferase activity was inhibited in the M. There were no treatment-related effects on O-deethylase activity. The results of the enzyme activity analyses are presented in the following tables (provided from the original NDA submission):

The mean (± S.D.) P450 contents and enzyme activities in male dogs were:

Dose [mg/kg]	P450 [pmol/mg]	ECOD [pkat/mg]	AND [pkat/mg]	GT [pkat/mg]
Control	564 ± 39	32.4 ± 5.4	31.1 ± 1.5	17.5 ± 0.4
120	475 ± 87	25.0 ± 3.3	40.2 ± 1.4	13.2 ± 1.7
Induction [%]	84	77	129	75
ANOVA p-values	not significant	not significant	0.001	0.012

Induction is expressed as 120 mg/kg vs. control ratio

The mean P450 contents and enzyme activities in female dogs were:

Dose [mg/kg]	P450 [pmol/mg]	ECOD [pkat/mg]	AND [pkat/mg]	GT [pkat/mg]
Control	443 ± 47	32.8 ± 11.0	33.7 ± 6.9	15.7 ± 1.6
120	566 ± 81	51.8 ± 14.1	55.7 ± 10.9	14.9 ± 3.3
Induction [%]	128	158	165	95
ANOVA p-values	not significant	not significant	0.042	not significant

Induction is expressed as 120 mg/kg vs. control ratio

Study title: CG5503: 52-Week Oral (Gavage) Toxicity Study in the Dog

**Key study findings:**

- The target organs were the CNS and cardiovascular system
- **Convulsions** at the HD in 2 F (80 mg/kg/d, exposure slightly less than that at the clinical MRHD on an AUC basis, and approximately 2 times the exposure on a Cmax basis)
  - Resulted in sacrifice *in extremis* in one of the dogs
  - Known effect of opioid receptor agonists
  - Reversal by naloxone supports relationship to opioid pharmacological effect
  - Not observed after the recovery period
- **QT, including QTc (Fridericia's and Van de Water's corrections) prolongation** at the HD
  - Observed throughout the study in nearly all of the HD M and F
  - Reversible; not observed after the 4-week recovery period
- Slight, minimal **decrease in partial thromboplastin time** in the HD M and F throughout the treatment period, not reversible (observed at end of recovery)
- Slight **increase in plasma sodium** at the HD, compared to controls but not to baseline measurements, and with no associated changes in other electrolytes
- Minimal to **slight focal gliosis with perivascular mononuclear cell infiltration in the medulla oblongata and/or pons** in 1 HD F and in 2 MD F and 1 MD M, without relationship to convulsions and considered to be spontaneous, in agreement with the pathologist
- No treatment-related effects on P450 content; however, dose-related **increases in O-deethylase activity** were observed in F and dose-related **increases in N-demethylase activity** were observed in M and F. **2-aminophenol glucuronyltransferase activity was decreased** in M and F.
- The NOAEL was 10 mg/kg/day
- The systemic exposure to the parent drug at the NOAEL (AUC = 23.3 mcg.h/ml in the males and 16.6 mcg.h/ml in the females) represented approximately **0.05 times** the clinical exposure ( $\approx 500$  ng.h/ml) at the MRHD of 600 mg/day.
- The peak plasma tapentadol concentrations (Cmax) at the NOAEL (6.8 mcg/L in M and 6.3 mcg/L in F) represented approximately 0.06 times the Cmax ( $\approx 118$  ng/ml) at the MRHD of 600 mg/day.
- Exposure to the O-glucuronide metabolite at the NOAEL represented approximately 1.5 times the clinical exposure at the MRHD, AUC basis
  - Demonstrates lower metabolic transformation to the glucuronide in dog, when compared to rat which showed considerably higher glucuronide exposures

Study no.: TP2441

Conducting laboratory and location:

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Date of study initiation: June 10, 2002

GLP compliance: Yes

QA report: yes (x) no ( )

Drug tapentadol (here also referred to as BN200 and/or CG5503), lot # (Batch CEWS112, and % purity: 98.3%

**Methods**

**Doses:** 0, 10, 30, and 80 mg/kg/day; the doses were selected based on the results of the 13-week study in Beagle dogs (See Study TP2415). The animal allocations are presented in the following table (provided from the original NDA submission):

Dose Level	Group 1	Group 2	Group 3	Group 4
	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	80 mg/kg/day
Male Numbers	1-6	7-10	11-14	15-20
Female Numbers	21-26	27-30	31-34	35-40

Dose levels are expressed in terms of the material as supplied

Animal Nos. 5, 6, 19, 20, 25, 26, 38 and 39 in Groups 1 and 4 were allocated to a 4-week recovery period following the treatment period.

**Species/strain:** Pure-bred Beagle dogs  vaccinated against distemper, leptospirosis, parainfluenza, contagious hepatitis, bordetella, canine papilloma virus, rabies and parvovirus)

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**Number/sex/group or time point (main study):** 4/sex/dose

**Route, formulation, volume, and infusion rate:** The test article was dissolved in tap water, and administered by oral gavage at 2 ml/kg, once daily. Dose formulations were analyzed using triplicate samples (2 ml/sample) on Day 1 and during Weeks 6, 26, 32, 45, and 52.

**Satellite groups used for toxicokinetics or recovery:** 2 additional dogs/sex/dose controls and HD for 4-week evaluation of reversibility

**Age:** 6-8 months

**Weight:** 7.7-11.7 kg

**Unique study design or methodology:** The dogs were housed in group pens, although separated during feeding and observations, and were fed 350 g/day pelleted standard  dog maintenance diet at 1h after dosing. The feed was removed from access after 3 hours. Tap drinking water was provided *ad libitum*. Microsomes were prepared from the livers of 3 males and females in the groups administered 0, 30, and 80 mg/kg/day CG5503, for analysis of P450 content, and N-dealkylation, O-dealkylation, and glucuronyltransferase activities.

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**Observation times:**

**Mortality:** Twice daily.

**Clinical signs:** Twice daily.

**Body weights:** Baseline and once weekly.

**Food consumption:** Baseline and once daily.

**Ophthalmoscopy:** Baseline, during Dosing Weeks 13, 25, 39, and 52, Recovery Week 5.

**EKG:** Before dosing and at 1 hour post dose at baseline and during Dosing Weeks 1, 13, 25, 39, and 51, and Recovery Week 5.

**Hematology:** Blood samples collected from the jugular vein at baseline and during Weeks 6, 13, 25, 39, 52 and recovery Week 5, from all fasted-overnight animals; standard parameters were evaluated.

**Clinical chemistry:** Blood samples collected from the jugular vein at baseline and during Weeks 6, 13, 25, 39, 52 and recovery Week 5, from all fasted-overnight animals; standard parameters were evaluated.

**Urinalysis:** Urine was collected using a catheter from all dogs during Weeks 6, 13, 25, 39, 52 and recovery Week 5; standard parameters were evaluated.

**Gross pathology:** All animals were examined, including decedents. The surviving dogs were examined after 52 weeks treatment and 4 weeks recovery.

**Organ weights:** The following organs were weighed, from all dogs at necropsy: adrenal glands, brain (including brainstem), heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes with epididymides, thymus, thyroid gland with parathyroid and uterus.

**Histopathology:** Adequate Battery: yes ( x ), no ( )

Peer review: yes ( ), no ( x )

Principal Investigator: [ ]

b(4)

The following organs and tissues were examined microscopically: adrenal glands, aorta, bone (femur with articular surface), bone marrow (sternum), brain (including medulla/pons, cerebral and cerebellar cortex), epididymides (fixed in Bouin's solution), esophagus, eyes (with optic nerve, Heidenhain's Susa solution), female mammary gland area, male mammary gland area, gallbladder, heart, kidneys with ureters, large intestine (cecum, colon, rectum), larynx, liver, lungs (with bronchi and bronchioles, infused with formalin), lymph nodes (retropharyngeal, mesenteric), ovaries, pancreas, Peyer's patches, pituitary gland, prostate gland, salivary glands (mandibular, parotid, sublingual), sciatic

nerve, skeletal muscle, skin, small intestine (duodenum, jejunum, ileum), spinal cord (cervical, mid-thoracic, lumbar), spleen, stomach, testes (fixed in Bouin's solution), thymus, thyroid gland including parathyroid gland, tongue, trachea, urinary bladder, uterus with cervix and oviducts, vagina and all gross lesions.

**Toxicokinetics:** Blood samples (4 ml from the jugular vein) were collected on Day 1 and in Weeks 26, 39, and 52, at 0.5, 1, 3, 6, 8, 12, and 24 hours after dosing

**Other:** Liver samples collected from all dogs at necropsy, for enzyme activity analysis.

**Results:**

**Mortality:** There was one death in a HD F (#40), which was sacrificed *in extremis* on Study Day 12 following observations of convulsions on 2 days (Days 4 and 12).

**Clinical signs:**

- **Convulsions** were observed in 2 HD (80 mg/kg/day) F
  - Days 4 and 12, 30 minutes after dosing in dog #40
    - resulting in sacrifice *in extremis*
    - dosing stopped from Days 5-7, resumed on Day 8
    - no abnormal necropsy findings in this dog
  - Days 178, 308, 316, 344, 345, and 358, starting 20-30 minutes after dosing, and lasting 5 hours in dog #37
  - Convulsions accompanied by paddling movements, muscle twitching, tremor, recumbency and decreased activity with deep respiration.
  - Reversed by naloxone administration
- Decreased activity and salivation daily throughout study at 30 and 80 mg/kg/day
- Frequent recumbency, vomiting, and tremor, and occasional whimpering at 30 and 80 mg/kg/day
- Fearfulness, occasionally at 80 mg/kg/day
- Clinical signs started at 15-30 minutes after dosing and lasted up to 5 hours.

The treatment-related clinical signs during the study are summarized in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	0 (Control)		10		30		80	
	GM	GF	GM	GF	GM	GF	GM	GF
Number of Animals								
Clinical Observations								
Convulsions	-	-	-	-	-	-	-	-
Decreased activity	-	-	-	-	-	+	+	-
Salivation	-	-	-	-	+++	++++	++++	++++
Tremor	-	-	-	-	+	+	-	-
Fearful	-	-	-	-	-	-	-	-
Recumbency	-	-	-	-	Yes	Yes	Yes	Yes
Vomiting	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Feces containing mucus	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

- = No noteworthy findings, + = Mild, ++ = Moderate, +++ = Marked

**Body weights:** Slight reduction in BW (-0.2 to -0.8 kg) at 80 mg/kg/day during first 2 study weeks. Body weights reduced in F at 10 (-1.0%), 30 (-4.0%), and 80 (-4.0%) mg/kg/day compared to controls, at end of study. The body weight evaluation results are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	0 (Control)		10		30		80	
	6M	6F	4M	4F	4M	4F	6M	6F
Number of animals								
Body weight (kg <sup>a</sup> )	10.7	9.9	+5.6	-1.0	0	-4.0	+0.9	-4.0

a) at the end of dosing period. For controls, means are shown. For treated groups, percentage differences from controls are shown.

**Food consumption:** Slight reduction in food consumption at the highest dose during the first 2 weeks of the study (-11% to -23% in M and -9% to -11% in F). At the end of the study, food consumption was reduced in the HD M and HD F, compared to controls. The results of the food consumption measurements are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	0 (Control)		10		30		80	
	6M	6F	4M	4F	4M	4F	6M	6F
Number of animals								
Food consumption (g/animal/day <sup>a</sup> )	330	295	0	+2.4	0	+4.1	-4.6	-5.4

a) at the end of dosing period. For controls, means are shown. For treated groups, percentage differences from controls are shown.

**Ophthalmoscopy:** There were no treatment-related effects.

**EKG:** Dose-related prolonged QT

- beginning at 1 hour after dosing in almost all HD animals
- observed throughout the study
- increased corrected QTc interval values at the HD (80 mg/kg/day), suggesting QT prolongation effect not related to heart rate fluctuations
- reversible, no treatment-related effects during and after the recovery period.
- no other treatment-related effects in the ECG evaluation

The results of the EKG observations for QT interval (in ms., ±S.D.) are presented in the following tables (provided from the original NDA submission):

**QT Interval Observation Summary: Males**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 30 MG/KG	GROUP 4 80 MG/KG
Pretest					
Q-T INTERVAL (ms)					
-----					
Pretest	MEAN	192	208	201	190
	ST. DEV.	15	10	9	13
	N	6	4	4	6

-----  
 Van de Nater's correction  
 -----

Pretest	MEAN	234	246	245	236
	ST.DEV.	11	7	6	8
	N	6	4	4	6

-----  
 Fridericia's correction  
 -----

Pretest	MEAN	239	253	253	245
	ST.DEV.	14	12	6	11
	N	6	4	4	6

Week 1

-----  
 Q-T INTERVAL (ms)  
 -----

Before dosing	MEAN	188	203	200	187
	ST.DEV.	15	13	8	10
	N	6	4	4	6
1h after dosing	MEAN	195	210	208	218
	ST.DEV.	20	14	15	24
	N	6	4	4	6

-----  
 Van de Nater's correction  
 -----

Before dosing	MEAN	234	240	243	237
	ST.DEV.	14	7	10	8
	N	6	4	4	6
1h after dosing	MEAN	239	238	243	254
	ST.DEV.	14	10	4	17
	N	6	4	4	6

-----  
 Fridericia's correction  
 -----

Before dosing	MEAN	242	245	250	249
	ST.DEV.	18	11	14	13
	N	6	4	4	6
1h after dosing	MEAN	248	242	248	259
	ST.DEV.	19	17	5	18
	N	6	4	4	6

Week 13

-----  
 Q-T INTERVAL (ms)  
 -----

Before dosing	MEAN	197	195	201	202
	ST.DEV.	14	25	10	29
	N	6	4	4	6
1h after dosing	MEAN	203	205	210	220
	ST.DEV.	8	31	12	20
	N	6	4	4	6

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Van de Water's correction

Before dosing	MEAN	232	232	236	241
	ST.DEV.	5	14	10	15
	N	6	4	4	6
1h after dosing	MEAN	232	234	237	254 *
	ST.DEV.	10	15	7	14
	N	6	4	4	6

Fridericia's correction

Before dosing	MEAN	235	236	240	247
	ST.DEV.	6	12	17	14
	N	6	4	4	6
1h after dosing	MEAN	234	237	239	259 *
	ST.DEV.	15	13	10	15
	N	6	4	4	6

Week 25

Q-T INTERVAL (ms)

Before dosing	MEAN	195	200	205	194
	ST.DEV.	8	14	25	19
	N	6	4	4	6
1h after dosing	MEAN	203	208	200	213
	ST.DEV.	8	22	16	20
	N	6	4	4	6

Van de Water's correction

Before dosing	MEAN	231	238	234	237
	ST.DEV.	7	6	9	4
	N	6	4	4	6
1h after dosing	MEAN	237	245	238	247
	ST.DEV.	5	12	9	13
	N	6	4	4	6

Fridericia's correction

Before dosing	MEAN	233	245	237	246
	ST.DEV.	9	13	12	6
	N	6	4	4	6
1h after dosing	MEAN	240	251	243	253
	ST.DEV.	8	12	10	16
	N	6	4	4	6

Week 39

Q-T INTERVAL (ms)

Before dosing	MEAN	183	198	209	186
	ST.DEV.	15	26	13	23
	N	6	4	4	6
1h after dosing	MEAN	188	195	213	207
	ST.DEV.	9	30	21	21
	N	6	4	4	6

Van de Water's correction

Before dosing	MEAN	225	231	235	225
	ST.DEV.	8	11	10	16
	N	6	4	4	6
1h after dosing	MEAN	230	230	249 *	241
	ST.DEV.	4	15	9	11
	N	6	4	4	6

Fridericia's correction

Before dosing	MEAN	227	234	238	227
	ST.DEV.	9	7	12	17
	N	6	4	4	6
1h after dosing	MEAN	236	232	255 **	247
	ST.DEV.	8	12	7	15
	N	6	4	4	6

Week 51

Q-T INTERVAL (ms)

Before dosing	MEAN	193	190	185	187
	ST.DEV.	8	8	19	16
	N	6	4	4	6
1h after dosing	MEAN	197	198	193	203 *
	ST.DEV.	8	10	15	9
	N	6	4	4	6

Van de Water's correction

Before dosing	MEAN	230	233	227	229
	ST.DEV.	10	12	10	8
	N	6	4	4	6
1h after dosing	MEAN	232	225	231	233
	ST.DEV.	8	11	9	4
	N	6	4	4	6

Fridericia's correction

Before dosing	MEAN	232	239	231	234
	ST.DEV.	14	17	10	9
	N	6	4	4	6
1h after dosing	MEAN	237	226	234	235
	ST.DEV.	10	14	9	6
	N	6	4	4	6

Following 4-Week Recovery

GROUP 1  
0 MG/KG

GROUP 4  
80 MG/KG

Q-T INTERVAL (ms)

Recovery	MEAN	195	210
	ST.DEV.	7	14
	N	2	2

Van de Water's correction

Recovery	MEAN	224	245
	ST.DEV.	2	6
	N	2	2

Fridericia's correction

Recovery	MEAN	223	249
	ST.DEV.	4	5
	N	2	2

\*/\*\*: Dunnett-test based on pooled variance sig. at 5% or 1% level.

QT Interval Observation Summary: Females

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 30 MG/KG	GROUP 4 80 MG/KG
<u>Pretest</u>					
Q-T INTERVAL (ms)					
Pretest	MEAN	200	198	205	199
	ST.DEV.	14	17	10	22
	N	6	4	4	6
Van de Water's correction					
Pretest	MEAN	244	236	241	239
	ST.DEV.	11	11	9	14
	N	6	4	4	6
Fridericia's correction					
Pretest	MEAN	255	240	245	246
	ST.DEV.	14	11	11	16
	N	6	4	4	6
<u>Week 1</u>					
Q-T INTERVAL (ms)					
Before dosing	MEAN	192	193	198	193
	ST.DEV.	10	5	13	19
	N	6	4	4	6
1h after dosing	MEAN	204	198	210	227 * *
	ST.DEV.	14	5	12	16
	N	6	4	4	6
Van de Water's correction					
Before dosing	MEAN	234	235	238	239
	ST.DEV.	11	7	10	11
	N	6	4	4	6
1h after dosing	MEAN	244	240	244	269 * * *
	ST.DEV.	13	11	11	13
	N	6	4	4	6
Fridericia's correction					
Before dosing	MEAN	240	241	243	248
	ST.DEV.	17	10	11	10
	N	6	4	4	6
1h after dosing	MEAN	251	247	248	282 * * *
	ST.DEV.	19	17	14	14
	N	6	4	4	6

Week 13

Q-T INTERVAL (ms)					
-----					
Before dosing	MEAN	202	189	190	190
	ST.DEV.	4	13	12	14
	N	6	4	4	5
1h after dosing	MEAN	210	205	210	226 *
	ST.DEV.	9	10	12	25
	N	6	4	4	5
-----					
Van de Water's correction					
-----					
Before dosing	MEAN	233	228	233	232
	ST.DEV.	8	7	10	14
	N	6	4	4	5
1h after dosing	MEAN	233	237	243	262 ** a
	ST.DEV.	16	10	4	16
	N	6	4	4	5
-----					
Fridericia's correction					
-----					
Before dosing	MEAN	236	231	237	242
	ST.DEV.	11	8	12	20
	N	6	4	4	5
1h after dosing	MEAN	235	240	246	272 ** a
	ST.DEV.	16	14	4	17
	N	6	4	4	5

Week 25

Q-T INTERVAL (ms)					
-----					
Before dosing	MEAN	197	190	188	186
	ST.DEV.	8	12	10	19
	N	6	4	4	5
1h after dosing	MEAN	203	188	193	203
	ST.DEV.	4	10	10	20
	N	6	4	4	5
-----					
Van de Water's correction					
-----					
Before dosing	MEAN	239	241	234	236
	ST.DEV.	7	12	12	15
	N	6	4	4	5
1h after dosing	MEAN	239	235	228	248
	ST.DEV.	8	10	3	14
	N	6	4	4	5
-----					
Fridericia's correction					
-----					
Before dosing	MEAN	245	255	241	249
	ST.DEV.	9	16	17	20
	N	6	4	4	5
1h after dosing	MEAN	243	243	229	259
	ST.DEV.	13	13	1	16
	N	6	4	4	5

Week 39

Q-T INTERVAL (ms)					
-----					
Before dosing	MEAN	197	194	180 *	190
	ST.DEV.	8	9	0	12
	N	6	4	4	5
1h after dosing	MEAN	197	189	193 *	196
	ST.DEV.	14	10	10	11
	N	6	4	4	5

Van de Water's correction

Before dosing	MEAN	239	230	227	232
	ST.DEV.	8	8	2	19
	N	6	4	4	5
1h after dosing	MEAN	239	230	235	238
	ST.DEV.	10	11	10	11
	N	6	4	4	5

Fridericia's correction

Before dosing	MEAN	245	232	233	243
	ST.DEV.	12	10	5	25
	N	6	4	4	5
1h after dosing	MEAN	247	235	241	248
	ST.DEV.	11	17	14	17
	N	6	4	4	5

Week 51

Q-T INTERVAL (ms)

Before dosing	MEAN	193	188	183	188
	ST.DEV.	8	10	10	11
	N	6	4	4	5
1h after dosing	MEAN	195	190	189	203
	ST.DEV.	16	8	14	20
	N	6	4	4	5

Van de Water's correction

Before dosing	MEAN	239	230	227	234
	ST.DEV.	7	5	9	8
	N	6	4	4	5
1h after dosing	MEAN	230 <sup>a</sup>	228	230	240
	ST.DEV.	5	8	12	18
	N	6	4	4	5

Fridericia's correction

Before dosing	MEAN	247	235	231	241
	ST.DEV.	9	7	11	11
	N	6	4	4	5
1h after dosing	MEAN	233 <sup>a</sup>	230	234	247
	ST.DEV.	5	10	14	20
	N	6	4	4	5

Following 4-Week Recovery Period

		GROUP 1 0 MG/RG	GROUP 4 80 MG/RG
Q-T INTERVAL (ms)			
Recovery	MEAN	200	180
	ST.DEV.	0	0
	N	2	2

Van de Water's correction

Recovery	MEAN	242	231
	ST.DEV.	3	1
	N	2	2

Fridericia's correction

Recovery	MEAN	249	242
	ST.DEV.	5	4
	N	2	2

\*\*\*: Dunnett-test based on pooled variance sig. at 5% or 1% level.  
 a/b: t-test significant at 5% or 1% level; before vs. after dosing.

**Hematology:** Partial thromboplastin time (PTT) was decreased in the HD M and F throughout the study, compared to controls. The PTT values are presented in the following tables (provided from the original NDA submission):

MALES

	Group Group Name Dose Level	Group 1 Control 0 mg/kg	Group 2 Low Dose 10 mg/kg	Group 3 Mid Dose 30 mg/kg	Group 4 High Dose 80 mg/kg
PTT sec					
WEEK -1	MEAN	19.25 <sup>a</sup>	21.63	18.77	17.82
	S.D.	1.41	1.47	0.49	2.12
	N	6	4	4	6
WEEK 6	MEAN	18.05 <sup>a</sup>	17.75	16.88	15.82 <sup>**</sup>
	S.D.	0.88	1.39	1.42	0.43
	N	6	4	4	6
WEEK 13	MEAN	20.78 <sup>a</sup>	20.88	19.88	18.05 <sup>**</sup>
	S.D.	1.65	1.07	0.88	0.91
	N	6	4	4	6
WEEK 25	MEAN	20.80 <sup>a</sup>	20.40	18.78	17.02 <sup>**</sup>
	S.D.	0.54	0.29	1.89	1.54
	N	6	4	4	6
WEEK 39	MEAN	19.23 <sup>a</sup>	20.13	18.42	16.12 <sup>**</sup>
	S.D.	0.55	1.49	1.15	0.76
	N	6	4	4	6
WEEK 52	MEAN	20.88 <sup>a</sup>	21.42	18.83	18.42
	S.D.	1.83	2.63	2.00	1.52
	N	6	4	4	6
RECOVERY WEEK 5	MEAN	23.35 <sup>a</sup>			24.85
	S.D.	2.76			0.52
	N	2			2

\*\* = p<0.01

FEMALES

Group Group Name Dose Level	Group 1 Control 0 mg/kg	Group 2 Low Dose 10 mg/kg	Group 3 Mid Dose 30 mg/kg	Group 4 High Dose 80 mg/kg
-----------------------------------	-------------------------------	---------------------------------	---------------------------------	----------------------------------

PTT	sec					
WEEK -1	MEAN	17.35 a	17.45	16.65	16.32	
	S.D.	0.96	0.82	0.58	1.43	
	N	6	4	4	5	
WEEK 6	MEAN	16.37 a	15.75	16.35	14.96	
	S.D.	1.02	0.89	0.96	0.74	
	N	6	4	4	5	
WEEK 13	MEAN	18.68ad	18.27	17.25	16.25**	
	S.D.	0.91	0.46	1.19	1.55	
	N	8	4	4	5	
WEEK 25	MEAN	17.68 a	17.17	17.00	16.32	
	S.D.	1.48	0.75	1.41	0.74	
	N	6	4	4	5	
WEEK 39	MEAN	16.08 a	16.40	15.75	15.40	
	S.D.	1.36	0.22	0.87	1.39	
	N	6	4	4	5	
WEEK 52	MEAN	18.63 a	18.92	18.50	16.88	
	S.D.	2.07	0.87	3.38	2.17	
	N	6	4	4	5	
RECOVERY WEEK 5	MEAN	22.70 a			21.10	
	S.D.	4.24			0.57	
	N	2			2	

\*\* = p<0.01

**Clinical chemistry:** Dose-related increase in sodium in M, but not in F in Weeks 25, 39, and 52, reversible during the recovery period

The treatment-related increases in plasma sodium in M are presented in the following table (provided from the original NDA submission).

MALES				
Group	Group 1	Group 2	Group 3	Group 4
Group Name	Control	Low Dose	Mid Dose	High Dose
Dose Level	0 mg/kg	10 mg/kg	30 mg/kg	80 mg/kg

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Wk*	mol/l					
WEEK -1	MEAN	148.82 a	148.05	148.88	148.00	
	S.D.	1.05	0.95	0.53	1.33	
	N	6	4	4	6	
WEEK 6	MEAN	143.95 k	144.69	144.00	146.82	
	S.D.	0.54	1.35	0.37	2.08	
	N	6	4	4	6	
WEEK 13	MEAN	144.25ad	144.88	142.69	147.63	
	S.D.	0.93	2.69	2.06	3.69	
	N	6	4	4	6	
WEEK 25	MEAN	145.83bd	147.13	146.57	150.28**	
	S.D.	0.54	0.82	0.40	2.34	
	N	6	4	4	6	
WEEK 39	MEAN	144.07bd	145.25	145.07	148.00**	
	S.D.	0.95	1.39	0.78	3.45	
	N	6	4	4	6	
-----						
WEEK 52	MEAN	146.15ad	149.99*	148.65*	149.45**	
	S.D.	0.86	1.68	0.98	1.87	
	N	6	4	4	6	
RECOVERY WEEK 5	MEAN	148.10 a			149.38	
	S.D.	1.41			1.55	
	N	2			2	

\* = p<0.05 \*\* = p<0.01

**Urinalysis:** No treatment-related effects on urinalysis parameters, at the end of the study.

**Gross pathology:** No treatment-related findings in the gross pathology examination.

**Organ weights:** No treatment-related effects on organ weights.

**Histopathology:** Focal gliosis (minimal to slight) with perivascular mononuclear cell infiltration were observed in the medulla oblongata and/or pons in Animals #37 (HD F), #32 (MD F) and #11 (MD M), and minimal perivascular mononuclear cell infiltration in the medulla oblongata without gliosis was observed in Animal # 14 (MD M). There was no clear association with the observations of convulsions (in HD female dogs #37 and #40). No information was provided on historical incidence of these findings for the laboratory. The microscopic findings are considered to be spontaneous, in agreement with the pathologist. There were no microscopic findings in the recovery dogs. The notable treatment-related histopathology findings are summarized in the following table (provided from the original NDA submission):

APPEARS THIS WAY  
ON ORIGINAL

Daily Dose (mg/kg)	0 (Control)		10		30		80	
	GM	GE	GM	GE	GM	GE	GM	GE
Number of Animals								
Histopathology (No. of animals affected)								
Medulla oblongata								
Perivascular mononuclear cell								
Infiltration, minimal-slight	0	0	0	0	2	1	0	1
Focal unilateral gliosis, minimal	0	0	0	0	1	1	0	0
Focal bilateral gliosis, slight	0	0	0	0	0	0	0	1
Pons								
Focal unilateral gliosis, minimal-slight								
	0	0	0	0	1	1	0	1
Perivascular mononuclear cell								
Infiltration, minimal-slight	0	0	0	0	1	0	0	1

**Toxicokinetics:** The results of the 52-week oral toxicokinetic evaluation in dogs are presented in the following table (provided from the original NDA submission):

APPEARS THIS WAY  
ON ORIGINAL

**Table: Summary on exposure to CG5503-base in dogs after repeated oral doses on Day 1 and in Weeks 26, 39 and 52**  
(mean values ± standard deviation (S.D.))

Dose	[mg/kg]	Males			Females		
		10	30	80	10	30	80
<b>Day 1</b>							
C <sub>max</sub>	[µg/L]	5.9	22.8	106	8.8	19.6	340
S.D.		± 3.9	± 10.7	± 90.7	± 6.5	± 6.25	± 549
AUC <sub>0-∞</sub>	[µg·h/L]	13.1	66.6	428	16.4	46.5	417
S.D.		± 3.3	± 25.2	± 480	± 6.4	± 17.3	± 513
<b>Week 26</b>							
C <sub>max</sub>	[µg/L]	3.9	32.1	85.3	4.9	50.1	92.6
S.D.		± 2.9	± 25.1	± 31.9	± 1.2	± 37.8	± 52.0
AUC <sub>0-∞</sub>	[µg·h/L]	20.1	74.2	235	18.1	76.8	212
S.D.		± 3.6	± 20.4	± 42.8	± 1.4	± 27.5	± 75.0
<b>Week 39</b>							
C <sub>max</sub>	[µg/L]	6.0	16.1	272	6.5	36.6	359
S.D.		± 2.9	± 3.4	± 287	± 2.2	± 39.7	± 238
AUC <sub>0-∞</sub>	[µg·h/L]	19.9	65.8	509	17.7	64.1	543
S.D.		± 6.3	± 17.9	± 359	± 5.8	± 43.9	± 315
<b>Week 52</b>							
C <sub>max</sub>	[µg/L]	6.8	49.0	145	6.3	31.4	221
S.D.		± 6.4	± 35.8	± 129	± 2.4	± 15.3	± 255
AUC <sub>0-∞</sub>	[µg·h/L]	23.3	142	303	16.6	61.2	407
S.D.		± 8.7	± 74.6	± 103	± 5.5	± 22.9	± 360

**Table: Summary on exposure to CG5503-glucuronide in dogs after repeated oral doses in Weeks 26 and 52**  
(mean values ± standard deviation (S.D.))

Dose	[mg/kg]	Males			Females		
		10	30	80	10	30	80
<b>Week 26</b>							
C <sub>max</sub>	[µg/L]	8141	20937	47993	6895	19699	31737
S.D.		± 2102	± 3870	± 8201	± 2433	± 5772	± 13836
AUC <sub>0-∞</sub>	[µg·h/L]	33074	76229	231252	22908	65076	150289
S.D.		± 7880	± 6710	± 30616	± 6805	± 15891	± 57276
<b>Week 52</b>							
C <sub>max</sub>	[µg/L]	8522	23363	48027	6604	28643	46821
S.D.		± 1808	± 6650	± 7380	± 2433	± 9415	± 21362
AUC <sub>0-∞</sub>	[µg·h/L]	32044	88313	224693	24138	84304	231142
S.D.		± 8146	± 18485	± 38779	± 7936	± 22197	± 100293

**Other:**

- No effects of 52-week oral tapentadol treatment on P450 content in dog
- Dose-related increases in O-deethylase activity were observed in F
- Dose-related increases in N-demethylase activity were observed in M and F
- Decreased 2-aminophenol glucuronyltransferase activity in M and F

**3.4.4. Genetic toxicology**

**Summary:** BN 200 (tapentadol HCl) was evaluated by the Sponsor in a standard battery of genetic toxicity studies. The studies included *in vitro* assays in *Salmonella typhimurium* and *Escherichia coli* (Ames Test, Reverse Mutation Assay, using both the plate incorporation test in Experiment I and pre-incubation test in Experiment II), and two independent Chromosome Aberrations Assays in Chinese Hamster V79 cells. Additionally, tapentadol was tested in the Chromosome Aberration Assay in rat bone marrow cells *in vivo*, and the Unscheduled DNA Synthesis assay in rat hepatocytes *ex vivo*.

Tapentadol was clastogenic in the first of two independent *in vitro* Chromosome Aberration studies in Chinese hamster V79 cells, resulting in a statistically significant increase in the incidence of structural chromosome aberrations at concentrations greater than 1000 mcg/ml in the presence of S9 mix. A second study, conducted to further explore the results of the positive findings in the Chromosome Aberration assay in V79 cells, revealed no increases in the frequencies of cells with aberrations at concentrations of up to the maximum concentration tested (1500 mcg/ml for 4 hours without metabolic activation with S9, and up to 1000 mcg/ml for 4 hours and 300 mcg/ml for 18 and 28 hours exposure with S9 mix).

No evidence was found of mutagenic potential by tapentadol in *Salmonella typhimurium* strains TA1537, TA 98, TA 1535, and TA100, and in *Escherichia coli* strain WP2: trp; uvrA in the Ames test using the plate incorporation and pre-incubation methods at concentrations of up to 5000 mcg/plate. Tapentadol was also negative in the *in vivo* assay for clastogenicity in male and female Wistar rat bone marrow cells at doses of up to the maximum tolerated dose (MTD) of 40 mg/kg IV for 24 and 48 hours. Evaluation of potential mutagenicity in the hepatocytes of rats given up to 35 mg/kg IV and 350 mg/kg PO (gavage) tapentadol in the Unscheduled DNA Synthesis assay revealed no DNA increased repair synthesis induced indicative of treatment-related DNA damage.

In conclusion, tapentadol was equivocal in the *in vitro* Chromosome Aberrations assay in Chinese hamster V79 cells, in the presence of metabolic activation with S9. The findings suggest potential clastogenicity by a metabolite of tapentadol HCl in the rat, from which the metabolic activating system (S9 mix) was obtained. The identity and production by human metabolism of the potentially genotoxic metabolite is not known.

**Study title:** Salmonella Typhimurium and Escherichia Coli Reverse mutation Assay with BN 200

**Key findings:**

- BN 200 (Tapentadol) was negative in the Ames Test, under the conditions of this study using both the plate incorporation and pre-incubation methods

**Study no.:** TP 1990/95

**Conducting laboratory and location:** [ ] b(4)

**Date of study initiation:** September 22, 1995

**GLP compliance:** Yes

**QA reports:** yes (x ) no ( )

**Drug BN 200 (Tapentadol Hcl), lot # (Batch) 06, and % purity:** 99%

**Methods**

**Strains/species/cell line:** Histidine dependent S. typhimurium strains TA1535, TA1537, TA98 and TA100 [ ] and tryptophan-independent E. coli strain WP2uvrA [ ] were used in this study. b(4)

**Doses used in definitive study:** 33.3 – 5000.0 mcg/plate with and without metabolic activation with S9 mix [ ] 500 mg/kg IP) induced S9 liver microsomal fraction from the livers of male Wistar rats [ ] using the plate incorporation method test in Experiment I and the pre-incubation test in Experiment II b(4)

**Basis of dose selection:** A pre-experiment for cytotoxicity and mutagenicity, using the plate incorporation method was conducted to select the concentrations for the main study. Strains TA98 and TA100 were exposed (in triplicate plates) to 8 concentrations of test article (3.3-5000 mcg/plate) with and without metabolic activation with S9 mix, at 37degC for 60 minutes and then plated for further incubation for 48 hours. Based on the results of this assay showing normal background growth at up to the highest concentration, the test article concentrations 33.3, 100.0, 333.3, 1000.0, 2500.0 and 5000 mcg/plate were chosen for the main study. The results of the pre-experiment for cytotoxicity are presented in the following table (provided from the original NDA submission):

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Substance	Concentration per plate [µg]	Revertants per plate			
		TA 98		TA 100	
		-	+	-	+
Negative control	-	23	33	125	155
Solvent control	-	28	38	147	154
4-NOPD	10.0	118	/	/	/
Sodium azide	10.0	/	/	901	/
2-aminoanthracene	2.5	/	419	/	937
Test article	3.3	34	45	165	143
	10.0	27	46	177	168
	33.3	28	40	166	167
	100.0	31	35	172	166
	333.3	31	48	163	155
	1000.0	32	52	145	164
	2500.0	28	46	158	164
	5000.0	33	43	143	193

Negative controls: Untreated and solvent-treated (aqua bidest) cells

Positive controls: Sodium azide (NaN<sub>3</sub>, 10 mcg/plate, for TA1535 and TA100), 4-nitro-o-phenylene-diamine (4-NOPD, 10 mcg/plate, for TA1537 and TA98), and methyl methane sulfonate (5 mcg/plate, for WP2uvrA in the absence of metabolic activation with S9, and 2-aminoanthracene, (2-AA, 2.5 mcg/plate except 10 mcg/plate in strain WP2uvrA, for TA1535, TA1537, TA98, TA100, and WP2uvrA) in the presence of S9.

b(4)

Incubation and sampling times: Experiment I (plate incorporation method): 100 mL bacterial suspension (cf. test system, pre-culture of the strains) mixed with 100 mL test article solution (or solvent negative control, or reference positive control solution), 500 mL S9 mix (for metabolic activation test) or S9 mix substitution buffer (test without metabolic activation), and 2000 mL overlay agar were poured onto minimal agar plates.

Experiment II (pre-incubation assay): 100 mL test solution, 500 mL S9 mix or S9 mix substitution buffer and 100 mL bacterial suspension were mixed in test tube, and shaken at 37degC for 60 minutes. 2.0 ml overlay agar was added at 45 degC after pre-incubation, and the mixture was plated using 3 plates per dose level including controls for further incubation for 48 hours at 37 degC.

The colonies were counted using an AUTOCOUNT and scored for cytotoxicity and reversion rates.

b(4)

**Results**

Study validity

- Triplicate cultures were tested
- Colonies counted using an AUTOCOUNT C system
- Background growth on the negative control and test plates confirmed in the main study
- Normal range of spontaneous reversion rates observed; spontaneous reversion frequencies are presented in the following table (provided from the original NDA submission):

Range of spontaneous reversion frequencies*				
1535	1537	98	100	WP2 uvrA
10 - 29	5 - 28	15 - 57	77 - 189	28-63

- Criteria for positive response: dose-related and reproducible increase in number of revertants, or reproducible increase for at least one test concentration, requiring a 2X increase in reversions for strains TA100 and WP2uvrA, and a 3X increase in reversions for strains TA1535, TA1537, and TA98 compared to spontaneous reversion rate.
- Positive response induced by the positive control reference mutagens.

Study outcome: There were no treatment-related increases in cytotoxicity and in numbers of revertants/plate in any of the strains tested and at any dose level up to 5000 mcg/plate in the presence and absence of metabolic activation with S9 mix, when compared to historical and negative control values using the plate incorporation test in Experiment I and the pre-incubation method in Experiment II. The positive control articles, 4-nitro-o-phenylene diamine (in TA 98 and TA 1537), 2-aminoanthracene (in all strains), sodium oxide (in TA 100 and TA 1535), and methylmethane sulfonate (in WP2uvrA) induced increases in the numbers of revertants/plate in each strain tested according to criteria for positive response, above, thus confirming the validity of the study. Slight test article toxicity at the highest concentrations in the pre-incubation assay does not appear to have interfered with the plate incorporation test.

The results of the Ames test using BN 200 (Tapentadol HCl) are presented in the following tables (provided from the original NDA submission):

**APPEARS THIS WAY  
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## without S9 mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		WP2 <i>uvrA</i>	
	I	II	I	II	I	II	I	II	I	II
Negative control	9	17	9	8	23	51	125	103	40	41
Solvent control	9	23	11	8	28	54	147	92	50	42
Positive control <sup>±</sup>	191	499	218	38	118	187	901	490	800	785
33.3	7	20	7	7	28	45	166	77	45	38
100.0	9	20	9	8	31	40	172	91	42	38
333.3	8	23	11	8	31	36	163	98	31	41
1000.0	12	20	13	9	32	29	145	104	28	32
2500.0	13	17	8	7	28	25	158	104	28	31
5000.0	10	8	11	3	33	15	143	77	22	19

## with S9 Mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		WP2 <i>uvrA</i>	
	I	II	I	II	I	II	I	II	I	II
Negative control	17	14	17	16	33	62	155	161	45	42
Solvent control	20	19	19	14	38	52	154	157	40	50
Positive control <sup>±</sup>	158	110	176	58	419	464	937	506	262	280
33.3	13	20	13	15	40	61	167	176	48	51
100.0	12	14	19	15	35	53	166	170	53	49
333.3	13	19	19	13	48	66	155	153	55	63
1000.0	12	17	13	13	52	49	164	178	43	44
2500.0	12	15	15	15	46	41	164	156	35	42
5000.0	13	13	15	5	43	29	193	126	37	25

<sup>±</sup> Sodium azide (10.0 µg/plate) strains TA 1535 and TA 100  
4-nitro-o-phenylene-diamine (10.0 µg/plate) strains TA 1537 and TA 98  
Methyl methane sulfonate (5 µl/plate) strain WP2 *uvrA*

<sup>±</sup> 2-aminoanthracene (2.5 µg/plate) strains TA 1535, TA 1537, TA 98, and TA 100  
2-aminoanthracene (10.0 µg/plate) strain WP2 *uvrA*

**Study title:** Chromosome Aberration Assay in Chinese Hamster V79 Cells *in vitro* with BN 200

**Key findings:**

- BN-200 was positive for clastogenicity in V79 cells at the concentrations of 1000-2000 mcg/ml (highest concentrations tested) in the presence of metabolic activation with S9
- Statistically significant increases in the number of structural chromosomal aberrations (exclusion gaps) were observed at 1500 mcg/ml at 18 hours in Experiment I, at 1000 mcg/ml at 28 hours in Experiment I and at 1000-2000 mcg/ml at 18 hours in Experiment II, in the presence of S9
- No treatment-related increases in chromosomal aberrations were observed in the absence of S9, and in the negative control cultures
- Study validity was adequately established

Study no.: TP 1976/95 ( ) Project 525802

Conducting laboratory and location: ( )

Date of study initiation: September 20, 1995

GLP compliance: Yes

QA reports: yes (x) no ( )

Drug BN-200, lot # 06, and % purity: 99%

b(4)

**Methods**

Strains/species/cell line: V79 Cells (Chinese Hamster cell line, ( ))

b(4)

Doses used in definitive study: Test article dissolved in MEM, with and without metabolic activation with S9 mix ( ) induced S9 liver microsomal fraction from male Wistar rats, prepared and mixed with cofactors according to Ames et al (1977); the concentrations tested are presented in the following table (provided from the original NDA submission):

b(4)

**APPEARS THIS WAY  
ON ORIGINAL**

Table 2: Chromosome aberration assay with BN-200; applied doses

Fixation interval	Experiment	Concentrations µg/ml					
		<b>without S9 mix</b>					
18 h	I	10.0	<b>30.0</b>	<b>100.0</b>	<b>300.0</b>	500.0	700.0
18h	II	30.0	<b>100.0</b>	200.0	<b>300.0</b>	375.0	<b>450.0</b>
28h	I			100.0	<b>300.0</b>	500.0	700.0
28h	II			<b>200.0</b>	300.0	375.0	450.0
<b>with S9 mix</b>							
18 h	I	<b>100.0</b>	300.0	500.0	<b>700.0</b>	1000.0	<b>1500.0</b>
18 h	II	750.0	<b>1000.0</b>	1250.0	<b>1500.0</b>	1750.0	<b>2000.0</b>
28 h	I			500.0	700.0	<b>1000.0</b>	1500.0
28 h	II			750.0	1000.0	1250.0	<b>1500.0</b>

evaluated experimental points were printed in bold letter

**Basis of dose selection:** pretest using XTT-assay and qualitative evaluation of cell density and morphology to indicate toxicity response, at 3.0-2500.0 mcg/ml (+S9 and -S9 mix) for cytotoxicity assay: V79 cells were treated with test article at 37degC. Solvent and blank controls were included in the assay. Cell number and cell morphology were evaluated at 4 h and at 18-20 hours after the start of treatment. In the XTT-assay, the cells were treated with test article for 18-20 hours followed by 4 hours incubation with XTT-labeling reagent for 4 hours. The absorption was read at 450 nm to evaluate cleavage of the yellow tetrazolium salt XTT to form orange formazan dye by hydrogenase activity in active mitochondria. Cell viability was reported as percent of solvent controls. Toxicity was observed at 300.0-2500.0 mcg/ml -S9, and at 1000.0-2500.0 mcg/ml +S9.

The results of the pre-test toxicity evaluation of BN-200 are presented in the following table (provided from the original NDA submission):

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Table 4: Cytotoxicity of BN-200 to cultures of Chinese hamster cell line V79.

A: without S9 mix			
Concentration µg/ml	absorbance 450/690 nm	(±) standard deviation	relative absorbance* % of solvent control
blank	0.170	0.005	0.0
solvent control	1.057	0.055	96.3
solvent control	1.091	0.066	100.0
3.0	1.057	0.054	96.2
10.0	1.008	0.105	90.9
30.0	0.948	0.070	84.5
100.0	0.994	0.072	89.4
300.0	0.933	0.066	82.8
500.0	0.585	0.067	45.1
1000.0	0.259	0.078	9.7
2500.0	0.172	0.005	0.3

B: with S9 mix			
Concentration µg/ml	absorbance 450/690 nm	(±) standard deviation	relative absorbance* % of solvent control
blank	0.169	0.004	0.0
solvent control	1.103	0.089	111.9
solvent control	1.004	0.132	100.0
3.0	0.965	0.106	95.4
10.0	1.024	0.063	102.4
30.0	1.001	0.052	99.7
100.0	0.976	0.039	96.7
300.0	0.924	0.062	90.4
500.0	0.816	0.054	77.4
1000.0	0.486	0.036	37.8
2500.0	0.189	0.013	2.3

$$* \text{ relative absorbance} = \frac{100 \times (\text{absorbance}_{\text{test article}} - \text{absorbance}_{\text{blanks}})}{(\text{absorbance}_{\text{solvent control}} - \text{absorbance}_{\text{blanks}})}$$

Negative controls: MEM solvent

Positive controls: Ethylmethanesulfonate (EMS, purity >98%, 600 mcg/ml) without metabolic activation, and cyclophosphamide (CPA, purity 98%, 0.71 mcg/ml) with metabolic activation

b(4)

Incubation and sampling times: V79 cells were exposed with and without S9 mix at 37degC. The treatment interval in the presence of metabolic activation was 4 hours and without metabolic activation the intervals were 18 and 28 hours.

-S9: The concentrations tested without S9 mix in the first experiment were 10-700 mcg/ml for 18 hours incubation and 100-700 mcg/ml for 28 hours. The concentrations tested without S9 mix in the second experiment were 30-450 mcg/ml for 18 hours and 200-450 mcg/ml for 28 hours.

+S9: The concentrations used with S9 mix in the first experiment were 100-1500 mcg/ml for 18 hours and 500-1500 mcg/ml for 28 hours. The concentrations used with S9 mix in the second experiment were 750-2000 mcg/ml for 18 hours and 750-1500 mcg/ml for 28 hours.

Colcemid (0.2 mcg/ml) was added to the cultures 15.5 and 25.5 hours after the start of treatment, and incubation continued for the remaining 2.5 hours. The cells were then incubated in hypotonic solution for 20 minutes, fixed with 3 + 1 methanol + glacial acetic acid and stained with Giemsa for microscopic examination for chromosomal breaks, fragments, deletions, exchanges and disintegrations.

## Results

### Study validity

- Number of aberrations in the negative and solvent controls within range of historical laboratory control data (0.00%-4.00%).
- Statistically significant increase in number of cells with structural chromosomal aberrations in the cells treated with positive control articles.
- Assay conducted in duplicate (2 parallel cultures per test), with 100 metaphases scored per culture for structural chromosome aberrations.
- The criteria for classification of the test article as mutagenic was a reproducible, statistically significant concentration-related increase in number of structural chromosomal aberrations, or a statistically significant, reproducible positive response in at least one test point.

Study outcome: The numbers of polyploid cells and mitotic indices, in Experiments I and II are presented in the following tables (provided from the original NDA submission):

**APPEARS THIS WAY  
ON ORIGINAL**

**Experiment I**

Table 5: Number of polyploid cells and mitotic index; fixation intervals 18 h and 28 h

Treatment group	conc. per ml	S9 mix	fixation interval	polyploid cells*				mitotic index**			
				culture		total	mean	absolute		mean	%***
				1	2			1	2		
Solv. control <sup>M</sup>		-	18 h	3	2	5	2.5	16.1	14.9	15.5	100.0
Pos. control <sup>PM</sup>	600.0 µg	-	18 h	3	0	3	1.5	8.3	11.1	9.7	62.6
Test article	30.0 µg	-	18 h	0	2	2	1.0	16.5	15.4	16.0	102.9
"	100.0 µg	-	18 h	1	3	4	2.0	7.7	8.9	8.3	53.5
"	300.0 µg	-	18 h	3	1	4	2.0	8.4	7.6	8.0	51.6
Solv. control <sup>M</sup>		+	18 h	1	2	3	1.5	14.0	15.1	14.6	100.0
Pos. control <sup>PM</sup>	0.71 µg	+	18 h	2	3	5	2.5	6.6	5.1	5.9	40.2
Test article	100.0 µg	+	18 h	2	3	5	2.5	12.4	13.7	13.1	89.7
"	700.0 µg	+	18 h	3	1	4	2.0	12.3	15.0	13.7	93.8
"	1500.0 µg	+	18 h	1	3	4	2.0	6.8	5.9	6.4	43.6
Solv. control <sup>M</sup>		-	28 h	1	1	2	1.0	14.2	15.6	14.9	100.0
Test article	300.0 µg	-	28 h	0	2	2	1.0	6.9	4.9	5.9	39.6
Solv. control <sup>M</sup>		+	28 h	1	4	5	2.5	16.3	12.3	14.3	100.0
Test article	1000.0 µg	+	28 h	3	3	6	3.0	7.5	8.4	8.0	55.6

\* The number of polyploid cells was determined in a sample of 100 cells per culture of each test group

\*\* The mitotic index was determined in a sample of 1000 cells per culture of each test group

\*\*\* For the positive control groups and the test article groups, the relative values of the mitotic index are related to the solvent controls

<sup>M</sup> MEM

<sup>PM</sup> EMS

<sup>CPA</sup> CPA

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**Experiment II****Table 8: Number of polyploid cells and mitotic index; fixation intervals 18 h and 28 h**

Treatment group	conc. per ml	S9 mix	fixation interval	polyploid cells*				mitotic index**			%***
				culture		total	mean	absolute		mean	
				1	2			1	2		
Solv. control <sup>v</sup>		-	18 h	4	5	9	4.5	18.6	18.3	18.5	100.0
Pos. control <sup>ww</sup>	600.0 µg	-	18 h	3	4	7	3.5	8.6	11.0	9.8	53.1
Test article	100.0 µg	-	18 h	3	0	3	1.5	15.9	18.0	17.0	91.9
"	300.0 µg	-	18 h	4	0	4	2.0	14.2	13.2	13.7	74.3
"	450.0 µg	-	18 h	4	3	7	3.5	5.8	6.3	6.1	32.8
Solv. control <sup>v</sup>		+	18 h	2	2	4	2.0	10.2	13.0	11.6	100.0
Pos. control <sup>ww</sup>	0.71 µg	+	18 h	4	0	4	2.0	16.0	14.1	15.1	129.7
Test article	1000.0 µg	+	18 h	1	3	4	2.0	16.9	15.1	16.0	137.9
"	1500.0 µg	+	18 h	1	0	1	0.5	14.1	13.5	13.8	119.0
"	2000.0 µg	+	18 h	3	5	8	4.0	7.7	11.7	9.7	83.6
Solv. control <sup>v</sup>		-	28 h	2	0	2	1.0	11.9	11.2	11.6	100.0
Test article	200.0 µg	-	28 h	1	3	4	2.0	10.2	14.3	12.3	106.1
Solv. control <sup>v</sup>		+	28 h	3	1	4	2.0	14.5	16.0	15.3	100.0
Test article	1500.0 µg	+	28 h	5	5	10	5.0	13.2	11.2	12.2	80.0

\* The number of polyploid cells was determined in a sample of 100 cells per culture of each test group

\*\* The mitotic index was determined in a sample of 1000 cells per culture of each test group

\*\*\* For the positive control groups and the test article groups, the relative values of the mitotic index are related to the solvent controls

<sup>v</sup> MEM<sup>ww</sup> EMS<sup>www</sup> CPA

The results of the evaluation of structural chromosome aberrations in Experiments I and II are presented in the following tables (table provided from the original NDA submission):

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ON ORIGINAL

Table 6: Structural chromosome aberrations Experiment I; fixation interval 18 h

slide no.	cells scored	% aberrant cells			aberrations											
		incl. gaps	excl. gaps*	with ex-changes	gaps		chromatid type				chromosome type				other	
					g	ig	b	f	d	ex	ib	if	id	ex	ma	cd
without S9 mix																
Solvent control: MEM																
1	100				0	0	0	0	0	0	0	1	0	0	0	0
2	100				1	0	0	1	0	0	0	0	0	0	0	0
1+2	200	1.5	1.0	0.0	1	0	0	1	0	0	0	1	0	0	0	0
Positive control: EMS 600.0 µg / ml																
1	100				3	0	7	1	0	8	0	1	0	0	0	0
2	100				1	0	6	1	0	11	0	0	0	0	0	0
1+2	200	15.0	14.0	9.0	4	0	13	2	0	19	0	1	0	0	0	0
Test article: 30.0 µg / ml																
1	100				2	0	0	1	0	0	0	0	0	0	0	0
2	100				0	0	0	1	0	0	0	0	0	0	0	0
1+2	200	2.0	1.0	0.0	2	0	0	2	0	0	0	0	0	0	0	0
Test article: 100.0 µg / ml																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				3	1	1	1	0	0	0	0	0	0	0	0
1+2	200	3.0	1.0	0.0	4	1	1	1	0	0	0	0	0	0	0	0
Test article: 300.0 µg / ml																
1	100				1	0	0	1	0	1	1	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	1.5	1.0	0.5	1	0	0	1	0	1	1	0	0	0	0	0
with S9 mix																
Solvent control: MEM																
1	100				0	0	0	1	0	0	0	1	0	0	0	0
2	100				2	0	1	2	0	0	1	0	0	0	0	0
1+2	200	4.0	3.0	0.0	2	0	1	3	0	0	1	1	0	0	0	0
Positive control: CPA 0.71 µg / ml																
1	100				4	0	5	4	0	10	1	1	0	0	0	0
2	100				5	0	6	6	0	6	3	0	0	0	0	0
1+2	200	20.5	17.5	7.5	9	0	11	10	0	16	4	1	0	0	0	0
Test article: 100.0 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	1	0	1	0	0	0	0
1+2	200	1.0	1.0	0.5	0	0	0	0	0	1	0	1	0	0	0	0
Test article: 700.0 µg / ml																
1	100				4	0	0	0	0	1	0	0	0	0	0	0
2	100				2	1	1	0	0	1	0	0	0	0	0	0
1+2	200	5.0	1.5	1.0	6	1	1	0	0	2	0	0	0	0	0	0
Test article: 1500.0 µg / ml																
1	100				3	0	2	9	0	2	0	0	0	0	0	0
2	100				1	0	5	0	0	3	1	0	0	1	1	0
1+2	200	12.5	10.5	2.5	4	0	7	9	0	5	1	0	0	1	1	0

\* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosomal type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps] only exchanges are recorded additionally in these cells), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 7: Structural chromosome aberrations Experiment I; fixation interval 28 h

slide no.	cells scored	% aberrant cells			aberrations											
		incl. gaps	excl. gaps*	with ex-changes	gaps				chromatid type				chromosome type			other
					g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
					without S9 mix											
Solvent control: MEM																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0	0
Test article: 300.0 µg / ml																
1	100				3	0	0	1	1	0	0	0	0	0	0	0
2	100				0	0	0	0	0	1	0	0	0	0	0	0
1+2	200	3.0	1.5	0.5	3	0	0	1	1	1	0	0	0	0	0	0
					with S9 mix											
Solvent control: MEM																
1	100				0	0	0	2	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1+2	200	1.5	1.5	0.0	0	0	1	2	0	0	0	0	0	0	0	0
Test article: 1000.0 µg / ml																
1	100				7	0	1	3	0	2	2	0	0	0	0	0
2	100				1	0	2	0	0	3	0	0	0	0	1	0
1+2	200	8.5	5.5	2.0	8	0	3	3	0	5	2	0	0	0	1	0

\* inclusive cells carrying exchanges

**Abbreviations**

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible, b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps] only exchanges are recorded additionally in these cells), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

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Table 9: Structural chromosome aberrations Experiment II; fixation interval 18 h

slide no.	cells scored	% aberrant cells			aberrations											
		incl. gaps	excl. gaps*	with ex-changes	gaps		chromatid type				chromosome type				other	
					g	ig	b	f	d	ex	ib	if	id	ex	ma	cd
<b>without S9 mix</b>																
<b>Solvent control: MEM</b>																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1+2	200	0.5	0.0	0.0	1	0	0	0	0	0	0	0	0	0	0	0
<b>Positive control: EMS 600.0 µg / ml</b>																
1	100				5	0	9	1	1	11	3	0	0	0	0	0
2	100				7	0	4	2	0	23	0	0	0	0	0	0
1+2	200	20.5	20.0	15.0	12	0	13	3	1	34	3	0	0	0	0	0
<b>Test article: 100.0 µg / ml</b>																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	1	1	0	0	0	0	0	0	0
1+2	200	0.5	0.5	0.0	1	0	0	1	1	0	0	0	0	0	0	0
<b>Test article: 300.0 µg / ml</b>																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	0.5	0.0	0.0	1	0	0	0	0	0	0	0	0	0	0	0
<b>Test article: 450.0 µg / ml</b>																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				3	0	0	2	0	0	0	0	0	0	0	0
1+2	200	2.0	0.5	0.0	3	0	0	2	0	0	0	0	0	0	0	0
<b>with S9 mix</b>																
<b>Solvent control: MEM</b>																
1	100				6	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1+2	200	3.0	0.0	0.0	7	0	0	0	0	0	0	0	0	0	0	0
<b>Positive control: CPA 0.71 µg / ml</b>																
1	100				3	0	2	1	0	7	3	0	0	0	0	0
2	100				1	0	2	3	0	7	1	1	0	0	1	0
1+2	200	14.0	13.0	7.0	4	0	4	4	0	14	4	1	0	0	1	0
<b>Test article: 1000.0 µg / ml</b>																
1	100				0	0	2	0	0	3	1	0	0	0	0	0
2	100				3	1	2	1	0	2	0	0	0	0	0	0
1+2	200	7.0	5.0	2.5	3	1	4	1	0	5	1	0	0	0	0	0
<b>Test article: 1500.0 µg / ml</b>																
1	100				0	0	3	4	0	0	2	0	0	0	0	0
2	100				0	0	3	1	0	2	0	2	0	0	0	0
1+2	200	7.5	7.5	1.0	0	0	6	5	0	2	2	2	0	0	0	0
<b>Test article: 2000.0 µg / ml</b>																
1	100				1	1	0	4	0	3	0	0	0	0	0	0
2	100				4	0	0	12	0	2	0	1	0	0	0	0
1+2	200	11.5	10.0	2.5	5	1	0	16	0	5	0	1	0	0	0	0

\* inclusive cells carrying exchanges

**Abbreviations**

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible, b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps] only exchanges are recorded additionally in these cells), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

The results of the biometry evaluation are presented in the following table (provided from the original NDA submission):

Statistical significance at the five per cent level ( $p < 0.05$ ) was evaluated by means of the chi-square test. Evaluation was performed only for cells carrying aberrations exclusive gaps.

Experiment I

	Solvent control versus	fixation interval	S9 mix	p-value
Test group	30.0 µg/ml	18 h	-	n.c.
"	100.0 µg/ml	18 h	-	n.c.
"	300.0 µg/ml	18 h	-	n.c.
"	100.0 µg/ml	18 h	+	n.c.
"	700.0 µg/ml	18 h	+	n.c.
"	1500.0 µg/ml	18 h	+	0.01 > p > 0.001*
"	300.0 µg/ml	28 h	-	0.1 > p > 0.05
"	1000.0 µg/ml	28 h	+	0.05 > p > 0.025*
Negative control versus Positive Control				
EMS	600 µg/ml	18 h	-	0.001 > p > 0*
CPA	0.71 µg/ml	18 h	+	0.001 > p > 0*

Experiment II

	Solvent control versus	fixation interval	S9 mix	p-value
Test group	100.0 µg/ml	18 h	-	0.9 > p > 0.1
"	300.0 µg/ml	18 h	-	n.c.
"	450.0 µg/ml	18 h	-	0.9 > p > 0.1
"	1000.0 µg/ml	18 h	+	0.01 > p > 0.001*
"	1500.0 µg/ml	18 h	+	0.001 > p > 0*
"	2000.0 µg/ml	18 h	+	0.001 > p > 0*
"	200.0 µg/ml	28 h	-	0.9 > p > 0.1
"	1500.0 µg/ml	28 h	+	0.001 > p > 0*
Negative control versus Positive Control				
EMS	600 µg/ml	18 h	-	0.001 > p > 0*
CPA	0.47 µg/ml	18 h	-	0.001 > p > 0*

n.c. = not calculated as the aberration rate is equal or lower than the control rate  
 \* aberration rate is statistically significant higher than the control rate

The definitive chromosomal aberration study results are presented below (tables provided from the original NDA submission):

Table 1: Summary of results of the chromosomal aberration study with BN-200

Experiment	S9 mix	Concentration of BN-200 in µg/ml	Polyploid cells	Mitotic index in % of control	Aberrant cells in %			
					incl gaps	excl. gaps*	exchanges	
I	18 h	-	30.0	1.0	102.9	2.0	1.0	0.0
		-	100.0	2.0	53.5	3.0	1.0	0.0
		-	300.0	2.0	51.6	1.5	1.0	0.5
II	18 h	-	100.0	1.5	91.9	0.5	0.5	0.0
		-	300.0	2.0	74.3	0.5	0.0	0.0
		-	450.0	3.5	32.8	2.0	0.5	0.0
I	28 h	-	300.0	1.0	39.6	3.0	1.5	0.5
II	28 h	-	200.0	2.0	106.1	1.5	1.0	0.0
I	18 h	+	100.0	2.5	89.7	1.0	1.0	0.5
		+	700.0	2.0	93.8	5.0	1.5	1.0
		+	1500.0	2.0	43.6	12.5	10.5**	2.5
II	18 h	+	1000.0	2.0	137.9	7.0	5.0**	2.5
		+	1500.0	0.5	119.0	7.5	7.5**	1.0
		+	2000.0	4.0	83.6	11.5	10.0**	2.5
I	28 h	+	1000.0	3.0	55.6	8.5	5.5**	2.0
II	28 h	+	1500.0	5.0	80.0	11.0	9.0**	0.0

\* inclusive cells carrying exchanges

\*\* Aberration frequency statistically significant higher than corresponding solvent control values

Aberrant cells in the solvent (negative) control groups: 0.0 % - 3.0 %

Aberrant cells in the positive control groups: 13.0 % - 20.0 %

**Summary:** In experiment I, the mitotic indices were 53.5% and 51.6% of control after treatment with 100 and 300 mcg/ml BN 200 respectively in the absence of S9 mix, and 55.6% and 43.6% control after treatment with 1000 and 1500 mcg/ml respectively in the presence of S9 mix, at 18 hours. In experiment II, the mitotic index in the absence of S9 was reduced to 32.8% of control value at 18 hours after treatment with 450 mcg/ml. There was no decrease in the mitotic index in experiment II in the presence of S9 mix at concentrations of 1000-2000 mcg/ml, measured at 18 hours.

The number of cells with structural chromosomal aberrations was significantly increased at 1500 mcg/ml (10.5%) BN 200 measured at 18 hours and at 1000 mcg/ml (5.5%) measured at 28 hours in experiment I, and at 1000 (5.0%), 1500 (7.5%) and 2000 (10.0%) mcg/ml measured at 18 hours and 1500 mcg/ml (9.0%) measured at 28 hours in experiment II, in the presence of S9 mix. The test article-related aberrations were in the form of gaps. There were no increases in the rate of polyploid metaphases by BN 200 compared to control rates in either experiment. Structural chromosomal aberrations were significantly increased by the positive control articles EMS and CPA (13.0%-20.0%).

**Study title:** In vitro Chromosome Aberration Test in Chinese Hamster V79 Cells with BN 200

**Key findings:**

- BN 200 (tapentadol HCl) was negative for clastogenicity in vitro in the Chromosome Aberrations Assay in Chinese hamster cells (V79 cells) at concentrations of up to 1500 mcg/ml in the absence of metabolic activation with S9 for 4 hours, and 1000 mcg/ml for 4 hours and 300 mcg/ml for 18 hours incubation in the presence of S9, under the conditions of this study.
- This study is considered to be valid, based on standard criteria for the assay.

**Study no.:** TP2448 ( Study 746501)

**Conducting laboratory and location:** [redacted] b(4)

**Date of study initiation:** September 4, 2002

**GLP compliance:** Yes

**QA reports:** yes (x) no ( )

**Drug BN-200, lot # Batch CEWS140, and % purity:** 97.7%

**Methods**

**Strains/species/cell line:** V79 Cells (Chinese Hamster cell line, [redacted]) b(4)

**Doses used in definitive study:** Test article dissolved in MEM, with and without metabolic activation with S9 mix ([redacted]) induced S9 liver microsomal fraction from male Wistar rats, prepared and mixed with cofactors according to Ames et al (1977); the concentrations tested are presented in the following table (provided from the original NDA submission): b(4)

Table 2: Doses applied in the Chromosome aberration test with BN200

Preparation Interval	Exposure period	Exp.	Concentration In µg/ml					
			without S9 mix					
18 hrs	4 hrs	I	125	250	500	750	1000	1500
18 hrs	18 hrs	II	100	200	300	400	500	750
28 hrs	28 hrs	II			300	400	500	750
			with S9 mix					
18 hrs	4 hrs	I	125	250	500	750	1000	1500
28 hrs	4 hrs	II	125	250	500	750	1000	1500

evaluated experimental points were printed in bold letters

**Basis of dose selection:** Pretest toxicity assay at concentrations of up to 2650 mcg/ml (10 mM exposure for 4 and 24 hours, maximum under OECD Guideline 473). BN 200 was cytotoxic at 1325 mcg/ml and above with and without S9 mix. The concentration of 1500 mcg/ml with and without S9 mix was selected for the high concentration in

Experiment I. The pretest for Experiment II resulted in reduced cell numbers at 662.5 mcg/ml and above for 24 hours, and therefore the high concentrations of 750 mcg/ml without S9 and 1500 mcg/ml with S9 were chosen for evaluation in Experiment II. The results of the cytotoxicity pre-test are presented in the following tables (provided from the original NDA submission):

Table 4: Cytotoxicity of BN200 to cultures of Chinese hamster cell line V79.

without S9 mix, 4 hrs exposure			with S9 mix, 4 hrs exposure		
Concentration in µg/ml	Number of cells	% of solvent control	Concentration in µg/ml	Number of cells	% of solvent control
Solvent control	1093	100	Solvent control	735	100
20.7	1314	121	20.7	635	114
41.4	1264	117	41.4	631	113
82.8	1302	120	82.8	612	110
165.6	1101	102	165.6	636	87
331.3	1296	120	331.3	461	63
662.5	753	69	662.5	413	56
1325.0	441	41	1325.0	159	22
2650.0	0	0	2650.0	4	1

without S9 mix; 24 hrs exposure

Concentration in µg/ml	Number of cells	% of solvent control
Solvent control	893	100
20.7	869	97
41.4	745	106
82.8	508	73
165.6	345	122
331.3	478	69
662.5	114	16
1325.0	2	6
2650.0	0	6

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Table 5: Number of cells in % of solvent control

without metabolic activation (S9 mix)					
Experiment I: 4 hrs exposure			Experiment II: continuous exposure		
Preparation interval	Concentration in µg/ml	Cells in % of solvent control	Preparation interval	Concentration in µg/ml	Cells in % of solvent control
18 hrs	125	104	18 hrs	100	87
	250	91		200	112
	500	117		300	21
	750	87		400	46
	1000	75		500	22
	1500	31		750	18
			28 hrs	300	33
				400	11
				500	9
				750	10

with metabolic activation (S9 mix)					
Experiment I: 4 hrs exposure			Experiment II: 4 hrs exposure		
Preparation interval	Concentration in µg/ml	Cells in % of solvent control	Preparation interval	Concentration in µg/ml	Cells in % of solvent control
18 hrs	125	99	28 hrs	125	102
	250	93		250	112
	500	88		500	106
	750	63		750	59
	1000	52		1000	21
	1500	11		1500	0

experimental groups evaluated for cytogenetic damage were printed in bold letters

**Negative controls:** Culture medium and Deionized water (vehicle solvent) in culture medium (10% v/v)

**Positive controls:** Ethylmethane sulfonate (EMS, >98% purity, 100-200 mcg/ml) without S9, and Cyclophosphamide (CPA, 98% pure, 0.7-1.0 mcg/ml)

**Incubation and sampling times:** The experimental schedule is presented in the following table (provided from the original NDA submission):

	without S9 mix			with S9 mix	
	exp. I	exp. II		exp. I	exp. II
Exposure period	4 hrs	18 hrs	28 hrs	4 hrs	4 hrs
Recovery	14 hrs	-	-	14 hrs	24 hrs
Preparation interval	18 hrs	18 hrs	28 hrs	18 hrs	28 hrs

**Results**

Study validity

- Number of aberrations in the negative and solvent controls within range of historical laboratory control data (0.00%-4.00%).
- Statistically significant increase in number of cells with structural chromosomal aberrations in the cells treated with positive control articles (table provided from the original NDA submission):

Test group Final concentration	Aberrant cells in % (excl. gaps) range	Test group Final concentration	Aberrant cells in % (excl. gaps) range
without S9 mix		with S9 mix	
EMS 100 - 1000 µg/ml	8.0 - 100.0	CPA 0.7 - 1.0 µg/ml	8.5 - 95.5

- Assay conducted in duplicate (2 parallel cultures per test), with 100 metaphases scored per culture for structural chromosome aberrations, except for evaluation of 50 metaphase plates for the positive control cultures in Experiment 1 with S9 mix and 200 metaphase plates scored for the solvent control and concentration 750 mcg/ml.
- The criteria for classification of the test article as clastogenic was a reproducible, statistically significant concentration-related increase in number of chromosomal aberrations, or a statistically significant, reproducible positive response in at least one test point, compared to historical control data. The historical laboratory control data are presented in the following table, for reference (provided from the original NDA submission):

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Chinese hamster V79 cell cultures (January 2001 to June 2002)

Without S9 mix										
		Aberrant cells (%)								
Test group Concentration	cells scored	Inclusive gaps			exclusive gaps			with exchanges		
		range	mean	calculated range*	range	mean	calculated range*	range	mean	calculated range*
Negative control										
Culture medium: MEM	46200	0.0-5.0	1.5	0.0-2.4	0.0-4.0	1.0	0.3-1.6	0.0-2.0	0.2	0.0-0.4
Deionised water 10 % (w/v)	15200	0.0-4.5	1.3	1.0-2.7	0.0-2.5	1.0	0.4-1.7	0.0-1.5	0.2	0.0-0.4
Org. solvents** 0.5 % (w/v)	31000	0.0-5.5	1.3	0.0-2.7	0.0-3.0	1.0	0.3-1.6	0.0-1.5	0.2	0.0-0.4
<b>Total</b>	<b>54400</b>	<b>0.0-5.5</b>	<b>1.7</b>	<b>0.0-2.6</b>	<b>0.0-4.0</b>	<b>1.0</b>	<b>0.3-1.6</b>	<b>0.0-2.0</b>	<b>0.2</b>	<b>0.0-0.4</b>
Positive control										
EMS 100-1000 µg/ml	48000	0.0-100.0	24.6	13.0-35.7	0.0-100.0	23.7	13.1-34.4	0.0-40.0	7.6	3.8-11.5
With S9 mix										
		Aberrant cells (%)								
Test group Concentration	cells scored	Inclusive gaps			exclusive gaps			with exchanges		
		range	mean	calculated range*	range	mean	calculated range*	range	mean	calculated range*
Negative control										
Culture medium: MEM	34000	0.0-7.0	1.3	0.0-2.8	0.0-4.0	1.2	0.5-1.9	0.0-2.0	0.3	0.0-0.6
Deionised water 10 % (w/v)	11000	0.0-5.5	1.3	1.0-2.7	0.0-4.0	1.2	0.5-1.9	0.0-1.5	0.3	0.0-0.6
Org. solvents** 0.5 % (w/v)	21500	0.0-5.5	1.9	1.0-2.9	0.0-3.5	1.3	0.5-2.0	0.0-2.0	0.3	0.0-0.7
<b>Total</b>	<b>66500</b>	<b>0.0-7.0</b>	<b>1.3</b>	<b>0.0-2.8</b>	<b>0.0-4.0</b>	<b>1.2</b>	<b>0.5-1.9</b>	<b>0.0-2.0</b>	<b>0.3</b>	<b>0.0-0.6</b>
Positive control										
CPA 0.7-1.0 µg/ml	34000	0.0-95.5	16.5	13.1-23.6	0.0-55.5	15.9	11.8-22.0	0.0-23.0	5.6	3.4-8.3

\* mean ± standard deviation

\*\* organic solvents: acetone, DMSO, ethanol, and tetrahydrofuran

Study outcome: The numbers of polyploid cells and mitotic indices in Experiments I and II are presented in the following tables (provided from the original NDA submission):

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Table 6: Number of polyploid cells and mitotic index; preparation interval 18 hrs with and without S9 mix

Treatment group	Conc. per ml	S9 mix	Exposure period/ Recovery	Polyploid cells*				Mitotic indices**			
				culture		total	%	absolute		mean	%***
				1	2			1	2		
Neg. control		-	4 / 14 hrs	9	13	22	2.2	14.0	15.2	14.6	100.0
Solv. control <sup>†</sup>	10 %	-	4 / 14 hrs	10	15	25	2.5	13.1	13.7	13.4	100.0
Pos. control <sup>†††</sup>	200 µg	-	4 / 14 hrs	7	9	16	1.6	14.0	13.3	13.7	93.6
Test item	750 µg	-	4 / 14 hrs	22	3	30	3.0	9.9	11.9	10.9	81.3
-	1000 µg	-	4 / 14 hrs	19	3	16	1.6	12.6	13.5	13.1	97.4
-	1500 µg	-	4 / 14 hrs	14	18	32	3.2	7.4	7.5	7.5	55.6
Neg. control		+	4 / 14 hrs	11	3	14	1.4	15.0	16.4	15.7	100.0
Solv. control <sup>†</sup>	10 %	+	4 / 14 hrs	7	10	17	1.7	16.5	17.1	16.8	100.0
Pos. control <sup>†††</sup>	0.7 µg	+	4 / 14 hrs	5	7	12	1.2	11.5	11.8	11.7	74.2
Test item	500 µg	+	4 / 14 hrs	9	9	18	1.8	11.8	11.9	11.4	67.9
-	750 µg	+	4 / 14 hrs	12	9	21	2.1	12.1	12.3	12.5	74.1
-	1000 µg	+	4 / 14 hrs	14	9	23	2.3	11.4	11.2	11.3	67.3

\* The number of polyploid cells was determined of each test group in a sample of 500 cells per culture  
 \*\* The mitotic index was determined in a sample of 1000 cells per culture of each test group in %  
 \*\*\* For the positive control groups, the relative values of the mitotic index are related to the negative controls; for the test item treatment groups the values are related to the solvent controls  
 † deionised water  
 †† EMS  
 ††† CPA

Table 9: Number of polyploid cells and mitotic index; preparation interval 18 and 28 hrs without S9 mix; preparation interval 28 hrs with S9 mix

Treatment group	Conc. per ml	S9 mix	Exposure period/ Recovery	Polyploid cells*				Mitotic indices**			
				culture		total	%	absolute		mean	%***
				1	2			1	2		
Neg. control		-	18 / - hrs	15	18	34	3.4	10.0	10.2	10.1	100.0
Solv. control <sup>†</sup>	10 %	-	18 / - hrs	3	12	20	2.0	11.9	6.6	10.3	100.0
Pos. control <sup>†††</sup>	100 µg	-	18 / - hrs	19	14	33	3.3	8.6	11.3	10.3	102.0
Test item	100 µg	-	18 / - hrs	14	10	24	2.4	10.5	10.3	10.4	101.5
-	200 µg	-	18 / - hrs	11	11	22	2.2	7.9	11.1	9.5	92.7
-	300 µg	-	18 / - hrs	14	16	30	3.0	5.4	4.7	5.1	49.3
Neg. control		-	28 / - hrs	13	17	30	3.0	15.1	17.0	16.1	100.0
Solv. control <sup>†</sup>	10 %	-	28 / - hrs	15	17	32	3.2	13.4	13.0	13.2	100.0
Pos. control <sup>†††</sup>	100 µg	-	28 / - hrs	13	17	30	3.0	14.4	13.5	14.0	87.2
Test item	300 µg	-	28 / - hrs	17	10	27	2.7	11.5	11.0	11.3	85.2
Neg. control		+	4 / 24 hrs	12	16	26	2.6	10.0	12.5	11.3	100.0
Solv. control <sup>†</sup>	10 %	+	4 / 24 hrs	15	17	33	3.3	13.0	13.5	13.3	100.0
Pos. control <sup>†††</sup>	1.0 µg	+	4 / 24 hrs	22	18	40	4.0	15.0	16.0	15.5	137.6
Test item	500 µg	+	4 / 24 hrs	15	19	34	3.4	12.9	11.2	12.1	90.6
-	750 µg	+	4 / 24 hrs	13	16	34	3.4	14.7	16.0	15.4	115.4
-	1000 µg	+	4 / 24 hrs	33	44	82	8.2	13.5	12.0	12.3	97.5

\* The number of polyploid cells was determined of each test group in a sample of 500 cells per culture  
 \*\* The mitotic index was determined in a sample of 1000 cells per culture of each test group in %  
 \*\*\* For the positive control groups, the relative values of the mitotic index are related to the negative controls; for the test item treatment groups the values are related to the solvent controls  
 † deionised water  
 †† EMS  
 ††† CPA

The incidence of structural chromosome aberrations observed in Experiments I and II are presented in the following tables (provided from the original NDA submission):

Table 7: Structural chromosome aberrations Experiment I;  
preparation interval 18 hrs without S9 mix; exposure period 4 hrs

Side no.	Cells scored	% Aberrant cells			Aberrations											
		incl. gaps	excl. gaps*	with ex-changes	gaps				chromatid type				chromosome type			
					g	ig	b	f	d	ex	ib	is	if	cx	ma	cd
without S9 mix																
<b>Negative control</b>																
1	100				1	0	1	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1+2	200	1.5	0.5	0.0	2	0	1	0	0	0	0	0	0	0	0	0
<b>Solvent control: deionised water 10 %</b>																
1	100				0	0	1	1	0	0	0	0	0	0	0	0
2	100				0	0	0	1	0	0	0	0	0	0	0	0
1+2	200	1.5	1.5	0.0	0	0	1	2	0	0	0	0	0	0	0	0
<b>Positive control: EMS 200 µg / ml</b>																
1	100				5	0	1	0	0	0	2	0	0	0	0	0
2	100				0	0	2	5	0	6	1	2	0	0	0	1
1+2	200	13.5	12.0	7.0	5	0	3	5	0	14	3	2	0	0	1	
<b>Test item: 750 µg / ml</b>																
1	100				2	0	1	0	0	0	0	0	0	0	0	0
2	100				5	0	0	0	0	0	1	1	0	0	0	0
1+2	200	4.0	1.5	0.0	7	0	1	0	0	0	1	1	0	0	0	0
<b>Test item: 1000 µg / ml</b>																
1	100				2	0	0	2	0	0	0	1	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1+2	200	3.0	1.5	0.0	3	0	0	2	0	0	0	1	0	0	0	0
<b>Test item: 1500 µg / ml</b>																
1	100				0	0	0	0	0	1	0	0	0	0	0	0
2	100				0	0	0	1	0	0	0	0	0	0	0	0
1+2	200	1.0	1.0	0.5	0	0	0	1	0	1	0	0	0	0	0	0

\* inclusive cells carrying exchanges

**Abbreviations**

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

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Table 10: Structural chromosome aberrations Experiment II:  
preparation interval 18 hrs without S9 mix; exposure period 18 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations											
		incl. gaps	excl. gaps*	with ex-changes	gaps				chromatid type			chromosome type				other
					g	ig	b	f	d	ex	lb	lf	ld	cx	ma	cd
without S9 mix																
<b>Negative control</b>																
1	100				2	0	1	0	0	0	0	1	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	2.0	1.0	0.0	2	0	1	0	0	0	0	1	0	0	0	0
<b>Solvent control: deionised water 10 %</b>																
1	100				1	0	2	0	0	0	0	2	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1+2	200	2.0	1.5	0.0	1	0	3	0	0	0	0	2	0	0	0	0
<b>Positive control: EMS 150 µg / ml</b>																
1	100				4	0	7	3	0	7	1	2	0	0	0	0
2	100				2	0	6	1	0	7	8	1	0	0	0	0
1+2	200	10.0	17.0	6.5	6	0	13	4	0	14	9	3	0	0	0	0
<b>Test item: 100 µg / ml</b>																
1	100				1	0	0	0	0	0	1	0	0	0	0	0
2	100				0	0	0	1	0	0	0	0	0	1	0	0
1+2	200	1.5	1.0	0.5	1	0	0	1	0	0	1	0	0	1	0	0
<b>Test item: 200 µg / ml</b>																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	1	0	0	0	0	0	0	0	0	0
1+2	200	1.0	0.5	0.0	1	0	1	0	0	0	0	0	0	0	0	0
<b>Test item: 300 µg / ml</b>																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	0.5	0.0	0.0	1	0	0	0	0	0	0	0	0	0	0	0

\* inclusive cells carrying exchanges

**Abbreviations**

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, lb = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

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Table 11: Structural chromosome aberrations Experiment II:  
 preparation interval 28 hrs without S9 mix: exposure period 28 hrs;  
 preparation interval 28 hrs with S9 mix: exposure period 4 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations											
		incl. gaps	excl. gaps*	with ex-changes	gaps				chromatid type				chromosome type			other
					g	ig	b	f	d	ex	lb	lf	ld	cx	ma	cd
without S9 mix																
Negative control																
1	100				0	0	0	0	0	0	1	0	0	0	0	0
2	100				2	0	0	1	0	0	1	0	0	0	0	0
1+2	200	2.5	1.5	0.0	2	0	0	1	0	0	2	0	0	0	0	0
Solvent control: deionised water 10 %																
1	100				2	0	0	0	0	1	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	1.5	0.5	0.5	2	0	0	0	0	1	0	0	0	0	0	0
Positive control: EMS 100 µg / ml																
1	100				0	0	2	1	0	4	1	0	0	1	0	0
2	100				0	0	1	2	0	3	0	2	0	0	1	0
1+2	200	9.0	9.0	4.0	0	0	3	3	0	7	1	2	0	1	1	0
Test item: 300 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1+2	200	0.5	0.0	0.0	1	0	0	0	0	0	0	0	0	0	0	0
with S9 mix																
Negative control																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1+2	200	1.0	0.0	0.0	2	0	0	0	0	0	0	0	0	0	0	0
Solvent control: deionised water 10 %																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	1	0	0	0	0	0	0
1+2	200	0.5	0.5	0.0	0	0	0	0	0	1	0	0	0	0	0	0
Positive control: CPA 1.0 µg / ml																
1	100				2	0	1	0	0	1	4	4	0	1	0	0
2	100				3	0	0	2	0	2	1	6	0	2	0	0
1+2	200	11.5	9.5	3.0	5	0	1	2	0	3	5	10	0	3	0	0
Test item: 500 µg / ml																
1	100				1	0	1	0	0	1	0	0	0	0	0	0
2	100				1	0	0	0	0	1	0	2	0	0	0	0
1+2	200	3.5	2.5	1.0	2	0	1	0	0	2	0	2	0	0	0	0
Test item: 750 µg / ml																
1	100				1	1	1	2	0	0	1	0	1	1	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	3.0	2.0	0.5	1	1	1	2	0	0	1	0	1	1	0	0
Test item: 1000 µg / ml																
1	100				3	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1+2	200	2.0	0.5	0.0	3	0	1	0	0	0	0	0	0	0	0	0

\* inclusive cells carrying exchanges

**Abbreviations**

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

The results of the biometric tests for Experiments I and II, using the Fisher's exact test on cells carrying aberrations exclusive of gaps are presented in the following tables (provided from the original NDA submission):

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Table 12: Biometry of Experiment I

Test group versus solvent control	Preparation interval	Exposure period	S9 mix	p-value	
Test group	750 µg/ml	18 hrs	4 hrs	-	n.c.
"	1000 µg/ml	18 hrs	4 hrs	-	n.c.
"	1500 µg/ml	18 hrs	4 hrs	-	n.c.
"	500 µg/ml	18 hrs	4 hrs	+	n.c.
"	750 µg/ml	18 hrs	4 hrs	±	n.c.
"	1000 µg/ml	18 hrs	4 hrs	+	n.c.
Positive control versus negative control					
EMS	200 µg/ml	18 hrs	4 hrs	-	< 0.001 <sup>2</sup>
CPA	0.7 µg/ml	18 hrs	4 hrs	+	< 0.001 <sup>2</sup>

Table 13: Biometry of Experiment II

Test group versus solvent control	Preparation interval	Exposure period	S9 mix	p-value	
Test group	100 µg/ml	18 hrs	18 hrs	-	n.c.
"	200 µg/ml	18 hrs	18 hrs	-	n.c.
"	300 µg/ml	18 hrs	18 hrs	-	n.c.
"	300 µg/ml	28 hrs	28 hrs	-	n.c.
"	500 µg/ml	28 hrs	4 hrs	±	0.061
"	750 µg/ml	28 hrs	4 hrs	±	0.108
"	1000 µg/ml	28 hrs	4 hrs	±	n.c.
Positive control versus negative control					
EMS	100 µg/ml	18 hrs	18 hrs	-	< 0.001 <sup>2</sup>
EMS	100 µg/ml	28 hrs	28 hrs	-	< 0.001 <sup>2</sup>
CPA	1.0 µg/ml	28 hrs	4 hrs	+	< 0.001 <sup>2</sup>

n.c. not calculated as the aberration rate is equal or lower than the control rate  
 \* aberration rate is statistically significant higher than the control rate

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 ON ORIGINAL

Table 8: Structural chromosome aberrations Experiment I; preparation interval 18 hrs with S9 mix; exposure period 4 hrs

Side no.	Cells scored	% Aberrant cells			Aberrations												
		incl. gaps	excl. gaps*	with ex-changes	gaps				chromatid type				chromosome type				other
					g	ig	b	f	d	ex	ib	is	ix	cx	ma	cd	
with S9 mix																	
Negative control																	
1	100				2	0	0	1	0	1	0	0	0	0	0	0	
2	100				1	0	0	1	0	1	0	0	0	0	0	0	
1+2	200	3.5	2.0	1.0	3	0	0	2	0	2	0	0	0	0	0	0	
Solvent control: deionised water 10 %**																	
1	200				0	1	3	1	0	0	1	0	1	0	0	0	
2	200				0	0	6	3	0	2	3	0	0	0	0	0	
1+2	400	4.5	4.25	0.5	0	1	9	4	0	2	4	0	1	0	0	0	
Positive control: CPA 0.7 µg / ml***																	
1	50				10	1	18	11	0	9	5	2	0	0	3	0	
2	50				5	0	10	5	0	6	5	2	0	0	1	0	
1+2	100	50.0	49.6	15.0	15	1	28	16	0	15	11	4	0	4	0	0	
Test item: 500 µg / ml																	
1	100				1	0	0	0	0	2	0	0	0	0	0	0	
2	100				1	0	0	0	0	0	0	0	0	0	0	0	
1+2	200	3.0	2.0	1.0	2	0	0	0	0	2	0	0	0	0	0	0	
Test item: 750 µg / ml**																	
1	200				0	0	3	3	0	0	1	0	0	0	0	0	
2	200				1	0	1	0	0	0	0	0	0	0	0	0	
1+2	400	2.25	2.0	0.0	1	0	4	3	0	0	1	0	0	0	0	0	
Test item: 1000 µg / ml																	
1	100				1	0	0	0	0	1	1	0	0	0	0	0	
2	100				0	0	0	0	0	0	0	0	0	0	0	0	
1+2	200	1.5	1.0	0.5	1	0	0	0	0	1	1	0	0	0	0	0	

\* inclusive cells carrying exchanges  
 \*\* 200 metaphase plates per culture were evaluated due to inhomogeneous results  
 \*\*\* 50 metaphase plates per culture were evaluated due to strong clastogenic effects

**Abbreviations**

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

**Summary:** BN 200 was cytotoxic, reducing cell numbers by ≥50% at 1500 mcg/ml for 4 hours without S9 mix, and at 1000 mcg/ml for 4 hours with S9 mix. The mitotic index was reduced at 300 mcg/ml, after 18 hours exposure without S9.

No statistically significant or biologically relevant increases in the numbers of cells with structural chromosomal aberrations were observed in two *in vitro* assays in V79 cells incubated with BN 200 at concentrations of up to 1500 mcg/ml for 4 hours and 300 mcg/ml for 18 and 28 hours without S9 mix and up to 750 mcg/ml for 4 hours with S9 mix, when evaluated at 8 and 28 hours after exposure. The rates of aberrant cells treated with BN 200 were within the historical range and the range observed in the solvent control cells, although the solvent control aberration rate was increased slightly above historical control range in the first of the two experiments. In agreement with the Sponsor, this observation is considered to be a biologically irrelevant outlier. There were no biologically relevant increases in rates of polyploid metaphases compared to controls, although there was a single increase at 1000 mcg/ml in the presence of S9 (8.2%) compared to solvent controls (1.7%-3.3%) in the first experiment, which is considered by this reviewer to be artifact in agreement with the Sponsor. This study was validated by significant and greater than historical control increases in cells with structural chromosome aberrations by EMS at 100 and 200 mcg/ml and CPA at 0.7 and 1.0 mcg/ml.

**Study title:** Chromosome Aberration Assay in Bone Marrow Cells of the Rat with BN 200

**Key findings:**

- BN 200 was negative for clastogenicity in the Chromosome Aberration Assay *in vivo* in rat bone marrow cells, under the conditions of this study
- Toxicokinetic parameters were not measured in this study, although the presence and severity of the clinical signs indicated systemic exposure to the test article
- Validity of the study was supported by appropriately selected doses using pre-test for toxicity to identify the MTD, appropriate methodology including preparation and administration of the test article, species and numbers of rats/sex/group, and tissue sampling and analysis, observation of statistically significant increase in chromosome aberrations in the cyclophosphamide (CPA, positive control article) treated rats, and comparisons with rates of aberrant cells in the historical and negative control rats

**Study no.:** 550300 (TP2009)

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** February 17, 2006

**GLP compliance:** Yes

**QA reports:** yes (x) no ( )

**Drug BN 200, lot # Batch No. 06, and % purity:** 99%

**Methods**

**Strains/species/cell line:** Male and female Wistar rats   ages 6-10 weeks, mean weights 191.6 g males and 172.5 g females, n=6/sex/group

b(4)

**Doses used in definitive study:** 0 (vehicle), 4, 12 or 40 mg/kg IV (tail vein) in the 24 hour evaluation, and 40 mg/kg in the 48 hour evaluation, at an injection rate of 5 ml/kg, 0.5 ml/minute.

**Basis of dose selection:** The doses were selected based on a pre-test for toxicity to identify the maximum tolerated dose that caused toxicity without mortality. Rats given BN 200 at 35 and 40 mg/kg IV showed reduced spontaneous activity (all treated rats, duration 48 hours), abdominal position (all treated rats, duration 6-48 hours), apathy (all treated rats, 6-48 hours), and convulsions (all treated rats at 10 minutes after treatment, duration up to 1 hour). A high dose of 40 mg/kg was selected based on these observations.

**Negative controls:** Vehicle: 0.9% NaCl solution given IV at 5 ml/kg.

**Positive controls:** Cyclophosphamide (CPA,   7.5 mg/kg IV in males, 12.5 mg/kg IV in females, 5 ml/kg, in 0.9% NaCl solution).

Incubation and sampling times: The rats were administered Colcemid [ ] 2 mg/kg) 2.5 hours before sacrifice to arrest cells in metaphase, and were sacrificed 24 or 48 hours after dosing with the test and control articles. Bone marrow cells were collected from the femurs, plated and stained with Giemsa for analysis of the metaphase cells. The cells were evaluated by light microscopy for chromosomal gaps, breaks, fragments, deletions, exchanges, and disintegrations. A minimum of 100 metaphases were evaluated per animal. Cytotoxicity was determined by the percent cells in mitosis per 1000 cells (mitotic index).

## Results

### Study validity:

- Criteria for positive results were a dose-related increase in the number of structural chromosomal aberrations and a statistically significant response in at least one test point
- Aberration rate of negative control (excluding gaps) was below 2%
- Statistically significant response to the positive control was observed

Study outcome: Clinical toxicity was observed in all treated rats, with deaths in 2 male and 1 female rat given the highest dose of 40 mg/kg/ IV.

The results of the chromosome aberration assessment are presented in the following table (provided from the original NDA submission):

Experimental group	dose mg/kg b.w.	preparation p. admin. hours	number of cells scored	% aberrant cells		mitotic index %
				incl. gaps	excl. gaps	
0.9% NaCl-solution	0	24	1000	0.4	0.2	5.48
BN 200	4	24	1000	0.8	0.7	7.10
BN 200	12	24	1000	1.3	1.0	7.48
BN 200	40	24	1000	0.9	0.7	6.50
CPA: (males)	7.5	24	1000	5.0	4.3	5.72
(females)	12.5			10.8	9.6	4.20
BN 200	40	48	1000	0.8	0.6	4.95

The aberration types in each 1000 cells are presented in the following table (provided from the original NDA submission; Group 1 = negative control at 24 hours; Group 2 = 4 mg/kg BN 200 at 24 hours; Group 3 = 12 mg/kg BN 200 at 24 hours; Group 4 = 40 mg/kg BN 200 at 24 hours; Group 5 = CPA at 24 hours; and Group 6 = 40 mg/kg BN 200 at 48 hours):

group	gap	iso-gap	break	iso-break	fragment	iso-fragment	deletion	multiple aberration *	exchange	chrom. disintegration**
1	2	0	1	0	0	1	0	0	0	0
2	1	0	1	3	4	1	0	0	0	0
3	3	0	3	3	5	1	0	0	0	0
4	2	0	0	1	5	0	0	1	1	0
5 (m)	3	0	7	1	10	2	0	1	8	0
5 (f)	10	0	19	2	8	6	1	11	29	0
6	2	0	1	2	4	0	0	0	0	0

\* More than 5 aberrations excluding gaps in one cell; exchanges (but no other aberrations) were recorded separately

\*\* Pulverization

A slight decrease in mitotic index compared to control was observed in the 48 hour exposure (40 mg/kg BN 200), and therefore BN 200 is considered to be only slightly cytotoxic in this assay. There were no biologically relevant or statistically significant increases in aberration frequency by BN 200 compared to the negative control value. There was one exchange figure and one multiple aberrant cell at the 40 mg/kg dose level (24 hours after treatment), that are considered to be spontaneous due to the single occurrences. BN 200 is considered to be negative for clastogenicity, having induced no chromosome mutations in the chromosome aberration test in rat bone marrow cells under the conditions of this assay (table provided from the original NDA submission).

**Study title:** *In vivo/in vitro* unscheduled DNA synthesis in rat hepatocytes with BN 200

**Key findings:**

- BN 200 was negative in the Unscheduled DNA Synthesis assay in rat hepatocytes, at up to maximum tolerated doses (MTD based on preliminary toxicity evaluation) of 35 mg/kg IV and 350 mg/kg PO (gavage) under the conditions of this study.
- This study is considered to be valid, based on standard criteria for the assay.

**Study no.:** 584900

**Conducting laboratory and location:** [ ]

b(4)

**Date of study initiation:** April 8, 1997

**GLP compliance:** Yes

**QA reports:** yes (x) no ( )

**Drug BN 200, lot # Batch No. 07, and % purity:** 99%

**Methods**

Strains/species/cell line: Male Wistar Hanlbm rats [ ages 6-10 weeks, mean weights 193 g, n=5/test group).

] ages 6-

b(4)

Doses used in definitive study: Test article was dissolved in 0.9% NaCl solution for intravenous (IV) administration at 5 ml/kg at 0.5 ml/minute, and orally (PO by gavage) at 10 ml/kg.

The following doses were administered (values represent animal identification numbers, table provided from the original NDA submission):

Test group	hours after treatment 2 h		hours after treatment 16 h	
	oral	intravenous	oral	intravenous
Vehicle control			11 - 15	
Test article 3.5 mg/kg b.w.				21 - 25
Test article 35 mg/kg b.w.		6 - 10	16 - 20	31 - 35
Test article 350 mg/kg b.w.	1 - 5		26 - 30	
Positive control			36 - 40	

Basis of dose selection: The doses were selected based on a preliminary toxicity study in which the rats were administered BN 200 orally and intravenously and evaluated for toxicity at 1 and 24 hours after dosing. Treatment with 40 mg/kg IV BN 200 resulted in reduced activity, tumbling, apathy, tremor, dyspnea, and deaths in two animals in the first hour after dosing. A dose of 35 mg/kg IV produced reduced activity, apathy, tremor, and bloody nose; no deaths were observed in this group. Oral dosing with BN 200 resulted in reduced activity, apathy and deaths at doses of 500 and 750 mg/kg. At the 350 mg/kg PO dose, reduced activity, eyelid closure, and apathy were observed. The maximum tolerated doses in the toxicity assay were 350 mg/kg PO and 35 mg/kg IV.

Negative controls: 0.9% NaCl solution vehicle

Positive controls: 2-acetylaminofluorene (2-AAF, [

] purity 99%, in dimethylsulfoxide/polyethylene glycol 400 (1+9), 100 mg/kg PO, 10 ml/kg).

b(4)

Incubation and sampling times: In the main experiment, the rats were administered BN 200 (tapentadol) by the intravenous and oral route, oral solvent control and oral positive control articles as described under Doses Used, above. The rats were sacrificed 2 (at 35 mg/kg IV and 350 mg/kg PO) and 16 (at 3.5 and 35 mg/kg IV and 35 and 350 mg/kg PO) hours after treatment. Primary hepatocytes were isolated from each animal for incubation, labeled with <sup>3</sup>HTdR (5 mCi/ml, specific activity 20 Ci/mmol), and incubated overnight. The cells were fixed with methanol: acetic acid on cover-slips, mounted and coated with photographic emulsion. The emulsion was developed after 12-14 days, and

the cells were fixed, stained with haematoxylin/eosin, and evaluated microscopically. Evaluation was conducted on 2 slides per animal/50 cells per slide.

## Results

### Study validity:

- Number of silver grains in the nuclear area was counted by automated counter
- 2 slides per animal/50 cells per slide were evaluated
- Criteria for positive response was an increase in mean number of net grains to greater than 5/cell at any test point
- Historical control values for negative controls = 5.63 nuclear grains (range 0.02-9.92) and -4.45 net grains (range -0.01 to -12.29)
- Historical control values for 2-AAF = 52.91 nuclear grains (range 18.02 to 107.84) and 37.72 net grains (range 8.38 to 92.43).

**Study outcome:** There was no significant effect of BN 200 on the viability of the hepatocytes at the doses tested. The percent viability ranged from 70% to 87% in all groups. The results of the UDS mutagenicity study in rat hepatocytes are presented in the following table (provided from the original NDA submission):

Group	Dose/route (mg/kg)	Period (h)	Grains per nucleus (mean ± SD)	Grains per cytoplasmic area (mean ± SD)	Net grains per nucleus (mean ± SD)
BN 200	350 PO	2	7.87 ± 3.57	13.23 ± 5.34	-5.36 ± 3.00
BN 200	35 IV	2	6.78 ± 3.38	10.80 ± 4.87	-4.02 ± 3.55
Solvent	0 PO	16	5.82 ± 2.64	9.54 ± 3.79	-3.72 ± 3.38
BN 200	35 PO	16	6.66 ± 3.09	10.72 ± 4.14	-4.06 ± 3.61
BN 200	3.5 IV	16	7.54 ± 3.07	12.40 ± 4.67	-4.86 ± 4.40
BN 200	350 PO	16	6.32 ± 3.49	9.08 ± 3.31	-2.76 ± 2.88
BN 200	35 IV	16	6.96 ± 3.11	12.48 ± 4.53	-5.50 ± 4.18
2-AAF	100 PO	16	27.25 ± 12.21	8.63 ± 3.76	18.61 ± 11.20

No UDS induction was observed in the hepatocytes of rats given BN 200 or vehicle control, at 2 and 16 hours after treatment. In comparison, the positive control article 2-AAF significantly increase the number of nuclear and net grain counts, indicating DNA repair synthesis in response to DNA damage.

### 3.4.5. Carcinogenicity

**Summary:** Tapentadol was negative for carcinogenicity in 104-week studies in mice and rats (see ExecCAC Meeting Minutes of August 26, 2008, Appendix 1).

Male and female CD-1 mice were administered tapentadol by oral gavage at doses of 50-200 mg/kg/day for 2 years, with dosing adjustments as described below. The results of histopathology examination showed a statistically significant increase in hepatocellular carcinomas in the HD males (8% incidence, 4/51 at 200 mg/kg/day compared to 1/51, 1/51, 0/51, and 0/51 at 0, 0, 50 and 100 mg/kg/day, respectively), that was found not

statistically significant by Agency statistical analyses for this common tumor type in mice. There were statistically significant trends for subcutis sarcoma in male mice. However, there were no statistically significant treatment-related increases in any dosed group compared to concurrent controls. Historical control data suggested that the tumor incidences are within the background for the strain in this laboratory.

The carcinogenicity study in mice used an adequate number of animals and parameters evaluated. The doses were based on the results of 13-week dose range-finding study in mice, in which the proposed MTD was estimated to be between the doses of 200 and 300 mg/kg/day. The dosing in the pivotal study followed ExecCAC recommendations for addition of a second HD group, dosed initially at 200 mg/kg/day for 13 weeks, with addition of a HD group given 300 mg/kg/day to determine if the higher dose could be tolerated for the next 13 weeks and subsequent dose reduction if excessive toxicity was observed (see ExecCAC letter of December 11, 2003). The HD male and female mice were terminated in Week 92, due to low survival. Dosing was terminated in the MD2 males from Week 100 and in the MD2 females from Week 99 to the end of the planned dosing period, due to excessive mortality (survival of 20). The 100 week duration of treatment is sufficient duration to detect neoplastic and pre-neoplastic changes indicating oncogenicity. No peer review of the histopathology examination was conducted. Historical control data were provided for comparison. Exposure at the high dose represented approximately 1.4 times the clinical exposure to the parent drug and approximately 8.4 times the clinical exposure to the glucuronide metabolite at the MRHD of 600 mg/day, on an AUC basis.

Male and female Wistar rats were administered oral tapentadol by admixture in the diet, at daily doses of 10-250 mg/kg/day for 104 weeks. The results of the histopathology evaluation showed slight non-statistically significant (compared to controls) increases in the incidence of hepatocellular adenomas in the high dose females (2/49 vs 0/50 in each of the control groups), and one additional hepatocellular carcinoma in the high dose male rats (2/50 vs. 1/50 in each of the control groups). Agency statistical analyses detected positive trends in the incidence in female rats in liver hepatocellular adenoma ( $p < 0.025$ , incidence 0/100, 0/50, 0/50, 1/50 and 2/50 at 0, 10, 50, 125, and 250 mg/kg/day, respectively). There was a statistically significant dose response for increased liver adenomas + carcinomas in the female rats, but no statistically significant increases over controls in any treated group. Historical control data suggested that the tumor incidences are within the background for the strain in this laboratory.

There was a minimal but statistically significant ( $p < 0.005$ ) increase in centrilobular hepatocellular hypertrophy in the MD2 (125 mg/kg/day) and HD (250 mg/kg/day) male and female rats. The histopathology findings in the liver are likely related to chronic, adaptive response associated with treatment-related increased metabolic enzyme activities, in agreement with the Sponsor. Increased follicular cell hypertrophy and focal follicular hyperplasia in the thyroid was observed in the HD females, probably resulting from chronic, enhanced liver enzyme activities and hypertrophy, in agreement with the Sponsor.

The carcinogenicity study in rats used an adequate number of animals and parameters evaluated. The animal caging, in groups of five instead of singly in this dietary study was substandard and may have contributed to the high variability in tapentadol exposure observed in the toxicokinetic analyses. Toxicokinetic evaluation confirmed exposure to the test article. The short half-life (0.5-1 hour) may also have played a role in the observed exposure variability, if the rats ingested study drug at variable times before TK sampling. The doses were acceptably chosen, based on the results of 13-week dose range-finding study in Wistar rats, in which the proposed MTD was estimated to be 250 mg/kg/day. The dosing in the pivotal study was consistent with ExecCAC recommendations and concurrence. Sufficient numbers of rats survived to the end of the 104-week treatment period to detect non-neoplastic and neoplastic changes to evaluate oncogenicity. Historical control values for the laboratory were provided. The high dose exposure represents approximately 0.7 times in the male rats and 2.7 times in the female rats the exposure to the parent drug associated with the maximum recommended human dose (MRHD) and approximately 27 times in the male and female rats the clinical exposure to the glucuronide metabolite at the MRHD of 600 mg/day, on an AUC basis.

**Study title:** CG5503: 104 Week Oral (Gavage) Administration Oncogenicity Study in the Mouse

**Key study findings:**

- CG5503 (tapentadol), administered by oral gavage for 92-104 weeks at up to the maximum tolerated dose of 200 mg/kg/day was negative for carcinogenicity in CD-1 mice, under the conditions of this study
- Statistically significant increase in mortality at 100 and 200 mg/kg/day in male mice
- Slight increase in incidence of hepatocellular carcinomas in the high dose males (statistically significant at  $p < 0.05$ ), with 4/51 (incidence 8%) at 200 mg/kg/day, compared to 1/51, 1/51, 0/51 and 0/51 at 0, 0, 50, and 100 mg/kg/day, respectively, compared to 2.46% incidence in the historical control tumor incidence data for this laboratory; however, this is considered to be a common tumor in mice and was found not significant ( $p = 0.63$  for the trend value and  $p = 0.021$  to  $0.558$  in 3 pairwise comparisons) by Agency statistical analyses
- Agency statistical analyses detected the following results:
  - Trend toward increased hepatocellular adenomas in the female mice
  - Statistically significant dose response for liver adenoma + carcinoma in the male mice, but no statistically significant differences from control in the pairwise comparisons
  - Trend toward subcutis sarcoma in male mice
  - Trend toward increased ovarian benign granuloma cell and luteoma tumors in female mice
- Steady state exposure at 200 mg/kg/day PO ( $AUC_{0-24} \approx 633$  ng.h/ml in F and 763 ng.h/ml in M, mean = 700 ng.h/ml) represents approximately 1.4 times the clinical exposure at the MRHD of 600 mg/day ( $AUC \approx 494$  ng.h/ml)

- Exposure to the glucuronide metabolite in the mice represented approximately 8.4 times the clinical exposure to the metabolite at the MRHD

Adequacy of the carcinogenicity study and appropriateness of the test model: The 2-year carcinogenicity study on tapentadol in CD-1 mice was conducted under GLP, received proper quality assurance inspections, and used an appropriate test model with adequate number of animals evaluated per dose. Standard parameters were measured, at appropriate time intervals. The oral gavage route of administration was used in agreement with the proposed indication for oral clinical treatment, and the vehicle, physiological saline was appropriate. The doses were supported by previous 13-week dose selection study results, and received concurrence by the ExecCAC for the starting dose range and mid-study dose escalation. Dual negative control groups were used. Although the mid-dose and high dose mice were terminated early (HD in Week 91, MDF in Week 99, and MDM in Week 100), the treatment period in these animals was sufficient to detect potential pre-, non-, and neoplastic changes in the histopathology examinations at 105 weeks after the start of dosing. Percent survival to the end of the intended dosing period was adequate for statistical evaluation. All animals including the premature sacrifices and mice found dead were examined microscopically. Histopathology was not peer reviewed. Toxicokinetic evaluation of systemic exposure to the parent drug and main glucuronide metabolite was conducted in an appropriate number of satellite animals and the results, demonstrating adequate exposure to both agents were submitted with the study report. Historical control data for the laboratory were provided for comparison.

Evaluation of tumor findings: There was a slight but statistically significant ( $p < 0.05$ , Sponsor analysis) increase in the incidence of hepatocellular carcinomas in the high dose males, with 4/51 (incidence 8%) at 200 mg/kg/day, compared to 1/51, 1/51, 0/51 and 0/51 at 0, 0, 50, and 100 mg/kg/day, respectively. In the historical control tumor incidence data for this laboratory, the incidence was 2.46% (23 of 934 male mice evaluated). Agency statistical trend analyses and pairwise comparisons for hepatocellular carcinomas in the males revealed no statistically significant increases. There were statistically significant trends for subcutis sarcoma in male mice. However, there were no statistically significant treatment-related increases in any dosed group compared to concurrent controls. There were no other treatment-related tumor findings in the mice. Historical control data suggested that the tumor incidences are within the background for the strain in this laboratory. Tapentadol can be considered to be negative for carcinogenicity in CD-1 mice, under the conditions of this study. The highest dose tested represented approximately 1.4 times the clinical exposure to the parent drug and approximately 8.4 times the exposure to the glucuronide metabolite at the MRHD of 600 mg/day tapentadol, on an AUC basis.

Study no.: TP2518 (C Study Number 1017/022)

Conducting laboratory and location: C

Date of study initiation: March 9, 2004

b(4)

**GLP compliance:** Yes

**QA report:** yes ( x ) no ( )

**Drug CG5503 (Tapentadol), lot # (Batch) E0001/10, and % purity:** 100.2%

**CAC concurrence:** Yes

**Methods**

**Doses:** The following dose schedule was used (table provided from the original NDA submission):

Group	Group description	Dose level (mg/kg/day)	Animal numbers			
			Main study		Satellite study	
			Male	Female	Male	Female
1	Control 1	0	1-51	307-357	613-624	724-735
2	Low	50	52-102	358-408	625-645	736-756
3	Intermediate (I)	100	103-153	409-459	646-666	757-777
4	Intermediate (II)	200*	154-204	460-510	667-690	778-801
5	High	200 (Wks 1-13)	205-255	511-561	691-714	802-825
		300 (Wks 14-28)	835-843#	844-852#		
		200 (Wks 29-91)**				
6	Control 2	0	256-306	562-612	715-723	826-834

# dose escalation animals were administered the increased dose levels at Week 14 and 27 in advance of the main study and satellite animals

\* as the number of surviving Group 4 males fell to 20 in Week 100, the remaining Group 4 males were retained off-dose for the remainder of the study

\*\* All surviving Group 5 animals were subject to early termination from the study in Week 92, since Group 5 reached a level considered insufficient for the continued viability of this group.

**Basis of dose selection:** MTD, based on 13-week dose selection study (Study 1017/021); the results showed convulsions and death in 1F at 300 mg/kg/day, one minute after dosing, and convulsions in 2F and 1M within 10 minutes of treatment at this dose, resulting in sacrifices *in extremis*. The remaining mice at the 300 mg/kg/day dose level showed hyperactivity. The results at the remaining doses of 10, 30, 100, and 200 mg/kg/day showed dose-related clinical chemistry changes and increased liver weights. The doses selected for the pivotal carcinogenicity study, based on these findings, were 50, 100 and 200 mg/kg/day. Agency recommended increasing the HD in the carcinogenicity study to 300 mg/kg/day after 13 weeks treatment at 200 mg/kg/day if tolerated. The HD was increased to 300 mg/kg/day in the carcinogenicity study after 13 weeks at the lower dose, but reduced to 200 mg/kg/day after increased mortality was observed with dose escalation.

b(4)

**Species/strain:** mice

**Number/sex/group (main study):** 51/sex/dose, total randomization procedure used for treatment group assignment.

b(4)

**Route, formulation, volume:** Test article dissolved in physiological saline (0.9% w/v NaCl) administered by oral gavage at 10 ml/kg/day

b(4)

**Frequency of dosing:** Once daily

**Satellite groups used for toxicokinetics or special groups:** 20/sex/dose for TK evaluation

**Age:** 6-7 weeks at start of dosing

**Weights:** 25.9-37.3 g males and 20.5-28.3 g females at the start of dosing

**Animal housing:** The mice were housed 3/cage, in a temperature (19degC-25degC), humidity (40%-70%), and air flow (15 changes/hour) controlled animal room, with 12-hour light-dark cycle.

**Restriction paradigm for dietary restriction studies:** None. The mice were provided food [ ] and water *ad libitum*. ]

b(4)

**Drug stability/homogeneity:** Formulation stability and homogeneity analyses performed (3 samples each from the top and bottom of each batch) in Weeks 1, 13, 14, 26, 39, 52, 65, 78, 91, and 104. The results showed concentration levels of 92%-105% nominal. Certificate of analysis provided;

**Dual controls employed:** Yes

**Interim sacrifices:** No.

**Deviations from original study protocol:** Dosing was terminated in the group 4 males from Week 100 to the end of the planned dosing period, due to excessive mortality in that group (survival of 20). The Group 5 male and female mice were terminated in Week 92, due to low survival. Group 5 animals were dosed at 400 mg/kg/day from Week 27 until Week 29, and then were returned to the 300 mg/kg/day dose after FDA review of data collected at 300 mg/kg/day.

**Observation times:**

**Mortality:** Checked twice daily

**Clinical signs:** Observations daily at 0 (immediately) after, and 0.25, 0.5, 1, 2, and 4 hours after dosing, with weekly detailed physical examinations including palpitation for tissue masses.

**Body weights:** Immediately prior to first dose, and weekly for 16 weeks, then once every 4 weeks until the end of the study.

**Food consumption:** Weekly for 16 weeks, and every 4 weeks thereafter until the end of the study.

**Ophthalmoscopic examination:** Baseline, and Weeks 52 and 104

**Clinical pathology:** Week 105; blood samples (0.5 ml) from 10 mice/sex/group, and all surviving HD animals in Week 91 for evaluation of standard hematology, bone marrow smears and clinical chemistry parameters.

**Histopathology:** Peer review: No. Study Pathologist [ ]

b(4)

Group 5 mice were terminated early in Week 91; dosing was terminated in the Group 4 male mice in Week 100 and Group 4 female mice in Week 99, but these animals were retained for sacrifice at the end of the intended dosing period. All animals, including premature sacrifices and mice found dead before the end of the study were included in the histopathology evaluation.

The following tissues were examined: adrenals, aorta, bone marrow smear (femur), brain cecum, colon, duodenum, eyes, femur with bone marrow and articular surface, gall

bladder, gross lesions, Harderian glands, head, heart, ileum, jejunum, kidneys, lacrimal glands, larynx, live, lungs with mainstem bronchi, mammary, mandibular lymph nodes, mesenteric lymph nodes, muscle (quadriceps), nasal cavities, nasopharynx, optic nerves, esophagus, ovaries with oviduct, pancreas, parotid gland, pituitary, preputial/clitoral gland, prostate, rectum, salivary glands, sciatic nerves, seminal vesicles, skin, spinal cord (cervical, lumbar, thoracic), spleen sternum with bone marrow, stomach, testes with epididymides, thymus, thyroids with parathyroids, tissue masses, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal glands.

**Toxicokinetics:** Blood (0.5 ml collected by orbital sinus puncture under halothane anesthesia) sampled from 3 satellite (TK) mice/sex/dose/time-point, at 0 (pre-dose), 0.25, 0.5, 1, 2, 5, and 8 hours after dosing, in weeks 4, 13, and 26, for analysis of plasma CG5503 and CG5503-glucuronide.

**Results**

**Mortality:** Dose-related increase in deaths during the study in the male and female mice, statistically significant in the MD1, MD2, and HD males and in the HD females compared to controls. The incidence of deaths during the dosing period (found dead and sacrificed *in extremis*), and percent survival at the end of the intended dosing period at 104 weeks are presented in the following table (provided from the original NDA submission):

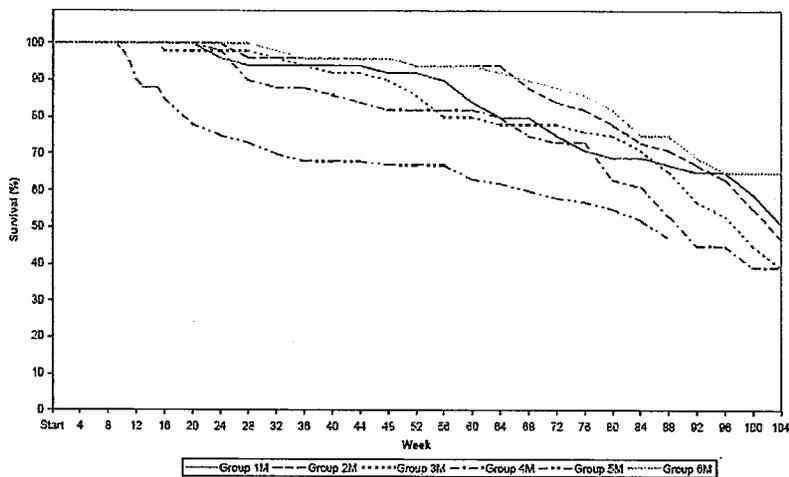
Daily dose (mg/kg) Sex	0 (Control)		0 (Control)		50		100		200	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Number of animals										
At start	51	51	51	51	51	51	51	51	51	51
Died or sacrificed moribund	25	35	18	37	27	34	31	30	31	34
Terminal sacrifice	26	16	33	14	24	17	20	21	20	17
Survival (%)	51	31	65	28	47	33	39	41	39	33

Group survival is presented in the following figures (provided from the original NDA submission):

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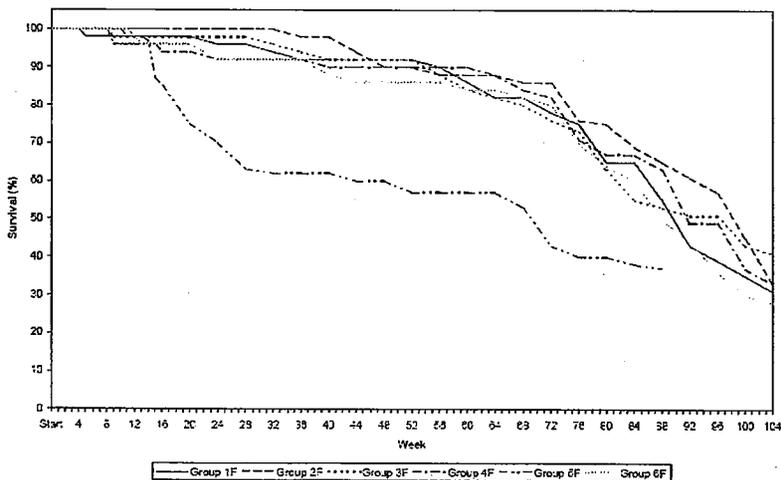
Group survival – males

Test Article	Control		D35503			Control
Group	1	2	3	4	5	6
Level (mg/kg/day)	0	50	100	200	200/300/200	0



Group survival – females

Test Article	Control		D35503			Control
Group	1	2	3	4	5	6
Level (mg/kg/day)	0	50	100	200	200/300/200	0



**Clinical signs:** No treatment-related clinical signs observed.

**Body weights:** No treatment-related effects up to 200 mg/kg/day, although there was a slight reduction in BWG in Group 5 males from Weeks 13-28 at 300 mg/kg/day. Group





ADRENAL	NUMBER EXAMINED:	24	27	31	32	27	18	35	34	30	35	34	37
NECROSIS/HEMORRHAGE		3	1	4	2	1	0	0	1	2	0	1	0
CORTICOTRUBULAR VACUOLATION		0	0	0	0	0	0	3	0	2	3	9	4
CORTICAL VACUOLATION		0	0	0	0	0	0	2	2	1	2	0	2
CORTICOTRUBULAR PIGMENT		3	3	1	1	1	1	21	22	17	13	9	18
CORTICAL ATROPHY		0	1	0	0	0	0	0	0	0	0	0	0
INFLAMMATORY CELL FOCI		0	0	0	2	0	0	1	0	0	1	0	0
HEMOPHAGES		0	0	0	0	0	0	2	1	0	3	1	0
HEMANGIOPLASIA		0	1	0	0	0	0	0	0	0	0	0	0
ANGIOMAS		0	0	0	0	0	0	0	1	0	0	0	0
POSTNATAL FOCUS		0	0	0	0	0	1	0	0	0	0	0	0
CLEAR CELL FOCUS		2	0	2	1	0	0	0	0	0	0	0	0
VACUOLATED FOCUS		0	0	0	0	0	1	0	0	0	0	0	0
MORPHOCHROMIC FOCUS		0	0	1	1	0	0	0	0	0	0	0	0
MELOBLASTIC HYPERPLASIA		0	3	0	0	1	0	0	0	0	0	0	0
SUBCAPSULAR CELL HYPERPLASIA		11	11	12	10	8	12	31	31	26	29	22	33

Group incidence microscopic data - non-neoplastic data - terminal kill

Test article	Control	GS503	Control
Group	1	2	3
Level (mg/kg/day)	0	50	100
			200
			300/300/200
			0

PRINTED: 05-OCT-07  
PAGE: 1  
STUDY NUMBER: 1017022

ORGAN AND FINDING DESCRIPTION	NUMBER	NUMBER OF ANIMALS AFFECTED											
		MALE						FEMALE					
		-1-	-2-	-3-	-4-	-5-	-6-	-1-	-2-	-3-	-4-	-5-	-6-
SPLLEN	NUMBER EXAMINED:	26	24	20	19	24	33	16	17	21	16	0	14
HEMOPHAGES		11	13	13	12	0	14	14	15	19	15	0	14
PIGMENT		2	1	2	0	0	1	7	6	8	6	0	3
ATROPHY		0	1	0	0	0	0	0	0	0	0	0	0
ANGIOMAS		0	0	0	0	0	0	0	1	0	0	0	0
HEMANGIOPLASIA		1	0	0	0	0	0	0	0	0	0	0	0
SPLENITIS		0	0	0	0	0	0	0	0	1	0	0	0
LYMPHOID HYPERPLASIA		14	11	10	7	0	12	9	5	3	10	0	7
STROMAL HYPERPLASIA		0	0	0	0	0	0	0	1	0	0	0	0
ADRENAL	NUMBER EXAMINED:	26	23	20	13	0	33	16	17	21	16	0	14
NECROSIS/HEMORRHAGE		0	0	0	0	0	0	0	0	1	0	0	0
CORTICOTRUBULAR VACUOLATION		1	0	0	0	0	0	0	1	0	0	0	0
CORTICAL VACUOLATION		0	0	0	0	0	0	0	0	0	1	0	1
CORTICOTRUBULAR PIGMENT		2	3	1	0	0	4	10	10	12	11	0	13
INFLAMMATORY CELL FOCI		0	0	0	0	0	2	6	0	0	0	0	0
POSTNATAL FOCUS		0	2	0	0	0	0	0	0	0	0	0	0
CLEAR CELL FOCUS		6	1	4	1	0	15	0	0	0	0	0	0
MORPHOCHROMIC FOCUS		9	3	1	1	0	2	0	1	0	0	0	0
BASOPHILIC FOCUS		0	1	1	0	0	0	0	0	1	0	0	0
MELOBLASTIC HYPERPLASIA		4	1	0	1	0	1	0	0	0	0	0	0
SUBCAPSULAR CELL HYPERPLASIA		15	11	11	10	0	19	16	17	19	16	0	14
MELOBLASTIC HYPERPLASIA - FOCAL		0	0	0	0	0	1	1	1	0	0	0	0

**Neoplastic:**

- Increased hepatocellular carcinomas in the males at the HD (statistically significant at 5% level by the Sponsor)
  - 4/51 (incidence 8%) at the HD of 200 mg/kg/day, compared to 1/51, 1/51, 0/51, 0/51, at 0, 0, 50 and 100 mg/kg/day, respectively
  - For reference, background (historical control) incidence of hepatocellular carcinoma in male CD-1 mice for the laboratory is 2.46% (23/934 males evaluated)
  - Agency statistical analyses showed no statistical significance in a trend test (p=0.063) and in the pairwise comparisons (p = 0.021-0.558 in 3 comparisons) for hepatocellular carcinoma in the male mice.
- Agency statistical analyses detected positive trends in the incidence of skin+subcutis sarcoma-NOS in male mice (p=0.0214 in the Peto test and p+0.054 in the poly-3 test).

The incidences of neoplastic findings are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	0 (Control)		0 (Control)		50		100		200	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Sex	51	51	51	51	51	51	51	51	51	51
<u>Number evaluated</u>										
<u>Number of animals with neoplastic lesions:</u>										
Skin and subcutis										
benign histiocytoma	0	0	0	0	0	0	0	1	0	0
squamous cell papilloma	0	0	0	0	0	0	1	0	0	0
benign hair follicle tumor	0	0	0	0	0	1	0	0	0	0
squamous cell carcinoma	0	1	0	0	0	0	0	0	0	0
malignant fibrous histiocytoma	0	0	1	0	0	0	0	0	0	0
osteosarcoma	0	0	1	0	0	0	0	0	0	0
sarcoma - NOS	1	0	0	0	2	0	1	0	3	0
Mammary gland: no examined	0	47	0	51	0	46	0	50	0	48
adenocarcinoma	-	3	-	1	-	3	-	1	-	1
Muscle: rhabdomyosarcoma	0	0	0	0	0	0	1	0	0	0
Femur: osteoma	0	1	0	0	0	0	0	0	0	0
Sternum: hemangioma	0	2	0	0	0	0	0	0	0	0
Liver										
hepatocellular adenoma	4	0	5	0	4	1	7	0	5	0
hemangioma	1	0	3	1	0	0	2	0	1	0
hepatocellular carcinoma	1	0	1	0	0	0	0	0	4	0
histiocytic sarcoma	1	1	0	1	0	0	0	1	1	0
hemangiosarcoma	1	0	0	0	1	0	0	0	1	0
Spleen: hemangioma	0	0	0	0	1	1	0	1	0	0
Pancreas: islet cell adenoma	0	0	0	1	0	0	0	1	0	1

Daily dose (mg/kg)	0 (Control)		0 (Control)		50		100		200	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Sex	50	51	51	51	51	49	49	50	48	49
Stomach no. evaluated										
adenoma	0	1	0	0	1	1	0	0	0	0
squamous cell papilloma	0	0	0	0	0	0	1	1	0	0
adenocarcinoma										
Duodenum no. evaluated	45	46	47	45	42	45	41	41	32	39
osteosarcoma	0	0	0	0	0	0	1	0	0	0
Adrenal no. evaluated	50	51	51	51	50	51	51	51	50	51
subcapsular cell adenoma	4	2	1	0	2	1	2	0	2	0
benign pheochromocytoma	0	0	0	0	0	0	1	0	0	0
Kidney no. evaluated	51	51	51	51	51	51	51	50	49	49
tubular cell adenomy	0	0	0	0	0	0	1	0	0	0
<u>Number evaluated</u>	51	51	51	51	51	51	51	51	51	51
Testes										
rete testis adenoma	0	-	0	-	0	-	1	-	0	-
interstitial cell adenoma	0	-	1	-	1	-	0	-	2	-
Ovary										
benign thecoma	-	0	-	1	-	0	-	0	-	0
benign luteoma	-	0	-	0	-	0	-	1	-	1
cystadenoma	-	2	-	2	-	2	-	0	-	1
Urinary bladder no. evaluated	51	50	51	50	50	50	48	48	48	45
transitional cell papilloma	0	0	0	0	0	0	0	0	1	0
Prostate: adenocarcinoma	1	-	0	-	0	-	0	-	0	-

Daily dose (mg/kg)	0 (Control)		0 (Control)		50		100		200	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
<u>Sex</u>	51	51	51	51	51	51	51	51	51	51
<u>Number evaluated</u>										
Uterus										
stromal polyp	-	3	-	3	-	4	-	3	-	1
leiomyoma	-	3	-	1	-	0	-	3	-	1
hemangioma	-	0	-	2	-	0	-	1	-	2
histiocytoma	-	1	-	0	-	0	-	0	-	0
adenocarcinoma	-	1	-	0	-	0	-	0	-	1
stromal sarcoma	-	0	-	0	-	0	-	1	-	1
leiomyosarcoma	-	0	-	1	-	0	-	0	-	0
hemangiosarcoma	-	0	-	0	-	1	-	0	-	0
histiocytic sarcoma	-	4	-	2	-	0	-	3	-	2
Uterus										
stromal polyp	-	0	-	1	-	2	-	0	-	0
stromal sarcoma	-	0	-	0	-	0	-	1	-	0
hemangiosarcoma	-	0	-	0	-	0	-	0	-	1
Lung										
bronchiolo-alveolar adenoma	9	3	11	7	11	7	7	6	12	7
bronchiolo-alveolar carcinoma	4	1	3	3	2	0	2	4	2	1
Tongue: squamous cell papillomas	1	0	0	0	0	0	0	0	0	0
Thyroid no. evaluated	50	51	50	51	49	51	49	49	51	51
follicular cell adenoma	1	0	1	0	0	0	0	0	0	0
Pituitary: adenoma	1	1	0	4	0	4	0	2	0	2
Brain: malignant meningioma	0	0	1	0	0	0	0	0	0	0
Daily dose (mg/kg)	0 (Control)		0 (Control)		50		100		200	
<u>Sex</u>	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
<u>Number evaluated</u>	51	51	51	51	51	51	51	51	51	51
Hem / lymph / retic										
malignant lymphoma-lymphoblastic	0	1	0	0	0	0	2	0	0	0
malignant lymphoma-pleomorphic	5	9	3	9	7	11	4	7	0	7
malignant lymphoma-lymphocytic	0	0	0	1	1	2	0	2	1	0
Connective tissue no. evaluated	1	4	2	3	3	5	1	0	2	0
histiocytic sarcoma	0	0	0	0	1	0	0	0	0	0
Tail no. evaluated	10	11	9	13	14	14	9	10	12	12
sarcoma - NOS	0	0	0	0	1	0	0	0	0	1
hemangiosarcoma	0	0	0	0	0	0	0	0	1	0
Oral cavity no. evaluated	0	0	0	0	0	0	0	1	0	0
osteosarcoma	0	0	0	0	0	0	0	1	0	0
Bone: osteoma										
Foot / leg no evaluated	2	0	0	0	0	0	3	1	0	1
squamous cell papilloma	1	0	0	0	0	0	0	0	0	0
fibroma	0	0	0	0	0	0	0	0	0	1
inflammatory myofibroblastic tumor	0	0	0	0	0	0	0	0	0	0
Harderian gland no. evaluated	0	0	0	0	2	0	0	0	0	1
adenoma	0	0	0	0	2	0	0	0	0	0
adenocarcinoma	0	0	0	0	0	0	0	0	0	1
Spinal column no. evaluated	1	1	0	0	1	0	0	0	0	0
osteosarcoma	0	0	0	0	1	0	0	0	0	0

Historical neoplasm data for male and female CD-1(ICR) mice in control groups from 104-week oral (gavage and dietary) studies conducted at [redacted] from 2000-2007 are presented in the following tables (provided from the original NDA submission):

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Incidence including merged tumour categories  
Control tumour incidence data - Carcinogenicity studies: 2000 - 2007  
Weeks: 104: Diet/Gavage dosed, Multiple housed, Ad-lib diet

Tissue	Merge category / tumour type	Incidence of individual tumours		Studies Combined Incidence				Minimum/Maximum Range across individual studies (%)					
		M	F	M	F	M	%	F	%	M Min	M Max	F Min	F Max
Abdominal cavity	M-sarcoma - nos	12	21	1	2	1	0.1	2	0.2	0.0	0.8	0.0	1.0
Adrenal		No. Exam: 928 980											
	B-cortical adenoma			1	0	1	0.1	0	0.0	0.0	1.0	0.0	0.0
	B-subcapsular cell adenoma			50	9	50	5.4	9	0.9	1.5	10.8	0.0	4.6
	<u>Adrenal: medullary tumours</u>					0	0.0	4	0.4	0.0	0.0	0.0	2.0
	B-benign pheochromocytoma			0	3								
	M-malignant neuroblastoma			0	1								
Bone		No. Exam: 9 19											
	B-ossifying fibroma			1	1	1	0.1	1	0.1	0.0	0.7	0.0	0.8
Brain		No. Exam: 931 986											
	M-malignant oligodendroglioma			0	1	0	0.0	1	0.1	0.0	0.0	0.0	0.8
	<u>Brain/spinal cord: meningeal tumours</u>					3	0.3	0	0.0	0.0	1.5	0.0	0.0
	B-benign meningioma			1	0								
	M-malignant meningioma			2	0								
		No. Exam: 817 921											
Caecum	B-leiomyoma			1	0	1	0.1	0	0.0	0.0	1.7	0.0	0.0
Colon		No. Exam: 879 957											
	<u>Colon: adenoma/carcinoma</u>					1	0.1	1	0.1	0.0	1.0	0.0	100.0
	B-adenoma			0	1								
	M-adenocarcinoma			1	0								
Connective tissue		No. Exam: 51 38											
	M-sarcoma - nos			0	1	0	0.0	1	0.1	0.0	0.0	0.0	0.8
Gall bladder		No. Exam: 800 887											
	B-papilloma			2	2	2	0.3	2	0.2	0.0	2.6	0.0	1.0

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Tissue	Merge category / tumour type	No. Exam:		Incidence of individual tumours				Studies Combined Tumour/merge category Incidence				Minimum/Maximum Range across individual studies (%)			
		M	F	M	F	M	%	F	%	M Min	M Max	F Min	F Max		
Lung		No. Exam: 935 987													
	<b>Lung: alveolar epithelial tumours</b>					232	24.8	160	16.2	20.3	34.5	12.4	24.8		
	B-bronchiolo-alveolar adenoma			159	120										
	M-bronchiolo-alveolar carcinoma			73	40										
Mammary gland		No. Exam: 13 862													
	<b>Mammary gland: epithelial tumours</b>					0	0.0	42	4.9	0.0	0.0	0.0	13.2		
	B-adenoma			0	4										
	B-fibroadenoma			0	2										
	M-adenocarcinoma			0	36										
Nasal cavity		No. Exam: 192 188													
	B-adenoma			1	0	1	0.5	0	0.0	0.0	0.8	0.0	0.0		
	B-ossifying fibroma			0	1	0	0.9	1	0.5	0.0	0.0	0.0	0.8		
Oesophagus		No. Exam: 934 987													
	M-leiomyosarcoma			1	1	1	0.1	1	0.1	0.0	1.0	0.0	0.7		
Tissue	Merge category / tumour type	No. Exam:		Incidence of individual tumours				Studies Combined Tumour/merge category Incidence				Minimum/Maximum Range across individual studies (%)			
		M	F	M	F	M	%	F	%	M Min	M Max	F Min	F Max		
Oral cavity		No. Exam: 2 3													
	B-squamous cell papilloma			1	1	1	0.1	1	0.1	0.0	1.0	0.0	0.7		
	M-malignant odontogenic tumour			0	1	0	0.0	1	0.1	0.0	0.0	0.0	1.4		
Ovary		No. Exam: - 882													
	B-cystadenoma			-	9	-	-	9	1.0	-	-	0.0	4.3		
	B-leiomyoma			-	1	-	-	1	0.1	-	-	0.0	1.4		
	<b>Ovary: sex cord/stromal tumours</b>							27	3.1	-	-	0.8	10.1		
	B-benign granulosa cell tumour			-	5										
	B-benign luteoma			-	7										
	B-benign sex cord stromal tumour			-	2										
	B-benign thecoma			-	2										
	B-tubulostromal adenoma			-	10										
	M-malignant granulosa cell tumour			-	1										
Pancreas		No. Exam: 932 980													
	<b>Pancreas: islet cell tumours</b>					5	0.5	6	0.6	0.0	2.6	0.0	2.9		
	B-islet cell adenoma			4	5										
	M-islet cell carcinoma			1	1										
Tissue	Merge category / tumour type	No. Exam:		Incidence of individual tumours				Studies Combined Tumour/merge category Incidence				Minimum/Maximum Range across individual studies (%)			
		M	F	M	F	M	%	F	%	M Min	M Max	F Min	F Max		
Penis		No. Exam: 31 -													
	M-basal cell carcinoma			1	-	1	3.2	-	-	0.0	0.7	-	-		
Pituitary		No. Exam: 919 973													
	B-adenoma			4	34	4	0.4	34	3.5	0.0	2.0	0.7	8.8		
Prostate		No. Exam: 930 -													
	<b>Prostate: adenoma/carcinoma</b>					2	0.2	-	-	0.0	1.0	-	-		
	B-adenoma			1	-										
	M-adenocarcinoma			1	-										
Seminal vesicle		No. Exam: 921 -													
	B-adenoma			1	-	1	0.1	-	-	0.0	0.8	-	-		

Tissue	Merge category / tumour type	No. Exam:		Studies Combined				Minimum/Maximum Range across individual studies (%)					
		M	F	Incidence of individual tumours		Tumour/merge category Incidence		M	M	F	F		
				M	F	M	%	F	%	Min	Max	Min	Max
Skin/appendage		935 987											
Skin + subcutis	B-sebaceous cell adenoma			1	1	1	0.1	1	0.1	0.0	1.0	0.0	0.7
Tail	B-neurofibroma			0	1	0	0.0	1	0.1	0.0	0.0	0.0	0.7
Tail	M-leiomyosarcoma			0	1	0	0.0	1	0.1	0.0	0.0	0.0	0.8
Tail	M-malignant histiocytoma			0	1	0	0.0	1	0.1	0.0	0.0	0.0	1.0
	<b>Skin/appendage: squamous cell tumours</b>					3	0.3	5	0.5	0.0	1.0	0.0	1.5
Ear	M-squamous cell carcinoma			1	0								
Foot/leg	B-squamous cell papilloma			1	1								
Skin + subcutis	B-benign hair follicle tumour			0	1								
Skin + subcutis	B-keratoacanthoma			1	0								
Skin + subcutis	B-trichoepithelioma			0	1								
Skin + subcutis	M-squamous cell carcinoma			0	2								
	<b>Skin/appendage: osteogenic tumours</b>					4	0.4	1	0.1	0.0	1.0	0.0	0.7
Foot/leg	M-osteosarcoma			1	0								
Skin + subcutis	M-osteosarcoma			3	1								
	<b>Skin/appendage: fibroblastic tumours</b>					15	1.6	12	1.2	0.0	3.3	0.0	3.3
Foot/leg	M-sarcoma - nos			1	1								
Skin + subcutis	M-fibrosarcoma			4	2								
Skin + subcutis	M-malignant fibrous histiocytoma			1	7								
Skin + subcutis	M-sarcoma - nos			9	2								
Tissue	Merge category / tumour type	No. Exam:		Studies Combined				Minimum/Maximum Range across individual studies (%)					
		M	F	Incidence of individual tumours		Tumour/merge category Incidence		M	M	F	F		
				M	F	M	%	F	%	Min	Max	Min	Max
	<b>Skin/appendage: basal cell tumours</b>					1	0.1	2	0.2	0.0	1.0	0.0	1.0
Skin + subcutis	B-benign basal cell tumour			1	0								
Skin + subcutis	M-malignant basal cell tumour			0	2								
	<b>Skin/appendage: lipomatous tumours</b>					1	0.1	1	0.1	0.0	1.5	0.0	1.4
Skin + subcutis	B-lipoma			0	1								
Skin + subcutis	M-liposarcoma			1	0								
Stomach		900 967											
	<b>B-adenoma</b>			1	3	1	0.1	3	0.3	0.0	0.9	0.0	1.5
	<b>Stomach: squamous cell tumours</b>					2	0.2	4	0.4	0.0	1.5	0.0	1.7
	B-squamous cell papilloma			1	3								
	M-squamous cell carcinoma			1	1								
Testis		934											
	B-interstitial cell adenoma			17	-	17	1.8	-	-	0.0	4.5	-	-
	B-rete testis adenoma			1	-	1	0.1	-	-	0.0	0.7	-	-
	M-malignant schwannoma			1	-	1	0.1	-	-	0.0	0.8	-	-
	M-sarcoma			1	-	1	0.1	-	-	0.0	1.4	-	-

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Tissue	Merge category / tumour type	Incidence of individual tumours		Studies Combined Tumour/mmerge category Incidence				Minimum/Maximum Range across individual studies (%)					
		M	F	M	F	M	%	F	%	M Min	M Max	F Min	F Max
<b>Blood vessel tumours continued</b>													
Tail	B-haemangioma			0	1								
Testis	B-haemangioma			3	0								
Urinary bladder	B-haemangioma			0	1								
Uterus	B-haemangioma			0	7								
<b>Histiocytic sarcoma</b>		No. Exam: 935 987				9	1.0	35	3.5	0.0	3.0	1.0	7.8
Abdominal cavity	M-histiocytic sarcoma			0	2								
Connective tissue	M-histiocytic sarcoma			2	1								
Epididymis	M-histiocytic sarcoma			2	1								
Foot/leg	M-histiocytic sarcoma			1	0								
Liver	M-histiocytic sarcoma			3	4								
Skin + subcutis	M-histiocytic sarcoma			0	3								
Tail	M-histiocytic sarcoma			1	0								
Thoracic cavity	M-histiocytic sarcoma			0	1								
Uterus	M-histiocytic sarcoma			-	23								
<b>Osteogenic tumours</b>		No. Exam: 935 987				11	1.2	14	1.4	0.0	5.0	0.7	2.9
Abdominal cavity	M-osteosarcoma			3	1								
Bone	B-osteoma			2	5								
Femur + marrow	B-osteoma			3	3								
Spinal cord	M-malignant osteosarcoma			0	1								
Bone	M-osteosarcoma			0	4								
Connective tissue	M-osteosarcoma			1	0								
Duodenum	M-osteosarcoma			1	0								
Tail	M-osteosarcoma			1	0								

**Toxicokinetics:** There was a dose-related increase in exposure (Cmax and AUC), but no change in exposure with increased duration of treatment from Weeks 4-26. There were no statistically significant increases in systemic exposure in the animals that received dose escalation from 200-300 mg/kg/day, in Week 16 compared to mice given 200 mg/kg/day for 26 weeks. The Tmax was 0.25-0.5 hour across dose groups. There were no gender-related differences in the toxicokinetic findings. No test article was found in the control vehicle-treated group. The results of the toxicokinetic evaluation are presented in the following table (provided from the original NDA submission):

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Mean exposure data as average  $C_{max}$  and  $AUC_{0-t}$  values ( $\pm$  SD) of CG5503 base:

Dose [mg/kg/day]	Time [Week]	$C_{max}$ [ng/mL]		$AUC_{0-t}^{(1)}$ [hng/mL]	
		Male	Female	Male	Female
50	4	148 $\pm$ 24	256 $\pm$ 20	144 $\pm$ 3	180 $\pm$ 19
	13	150 $\pm$ 45	296 $\pm$ 28	148 $\pm$ 40	201 $\pm$ 7
	26	114 $\pm$ 11	205 $\pm$ 47	145 $\pm$ 76	164 $\pm$ 23
	all weeks	137 $\pm$ 31	253 $\pm$ 49	146 $\pm$ 43	182 $\pm$ 23
100	4	472 $\pm$ 290	353 $\pm$ 149	315 $\pm$ 55	310 $\pm$ 93
	13	490 $\pm$ 332	265 $\pm$ 79	439 $\pm$ 163	291 $\pm$ 56
	26	467 $\pm$ 126	238 $\pm$ 75	315 $\pm$ 26	254 $\pm$ 58
	all weeks	477 $\pm$ 229	285 $\pm$ 106	356 $\pm$ 107	285 $\pm$ 66
200	4	758 $\pm$ 130	252 $\pm$ 63	674 $\pm$ 66	525 $\pm$ 205
	13	1117 $\pm$ 930	414 $\pm$ 146	874 $\pm$ 254	534 $\pm$ 135
	26	828 $\pm$ 382	610 $\pm$ 200	763 $\pm$ 286	633 $\pm$ 190
	all weeks	901 $\pm$ 533	425 $\pm$ 201	770 $\pm$ 213	564 $\pm$ 164

<sup>(1)</sup> t = 5, 8 or 24 hours depending on dose and treatment week

There was a dose-related increase in exposure ( $C_{max}$  and AUC) to the glucuronide metabolite. No accumulation was observed; no changes in exposure were found with increasing duration of treatment, from Weeks 4 to 26. The  $T_{max}$  for the metabolite was observed at 0.25-0.5 hour after dosing, across dose groups. No metabolite was found in the vehicle control groups. However, exposure to the metabolite was greater (factor 1.5-1.7) in the male than in the female mice. No increase in  $C_{max}$  (males and females) and AUC (males) was found between the mice that received dose escalation from 200 to 300 mg/kg/day CG5502, compared to the mice given 200 mg/kg/day for 26 weeks. The  $AUC_{0-24}$  was higher in the females that received 300 mg/kg/day when compared to female mice that received 200 mg/kg/day in Week 16, however. The results of the toxicokinetic evaluation on CG5503 glucuronide are presented in the following table (provided from the original NDA submission):

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Dose [mg/kg/day]	Time [Week]	C <sub>max</sub> [ng/mL]		AUC <sub>0-24h</sub> [h·ng/mL]	
		Male	Female	Male	Female
50	4	38429 ± 10364	22152 ± 650	44242 ± 9907	25241 ± 3185
	13	39335 ± 4336	25433 ± 6327	47750 ± 5234	29395 ± 1021
	26	34907 ± 3823	23489 ± 4257	46188 ± 1872	29046 ± 3441
	all weeks	37557 ± 6270	23692 ± 4085	46060 ± 5880	27894 ± 3121
100	4	39772 ± 6133	31633 ± 4298	58561 ± 3473	49646 ± 4972
	13	60944 ± 8700	24532 ± 3281	98083 ± 15901	52185 ± 11652
	26	48626 ± 4243	22580 ± 2283	82053 ± 12839	59234 ± 1406
	all weeks	49780 ± 10846	26248 ± 5063	79565 ± 20094	53688 ± 7689
200	4	60858 ± 2250	32650 ± 11519	165932 ± 21625	110405 ± 25368
	13	69977 ± 14790	25087 ± 2578	160099 ± 16408	79955 ± 11120
	26	57553 ± 10539	34686 ± 11171	177921 ± 22514	100115 ± 19481
	all weeks	62796 ± 10713	30808 ± 9231	167984 ± 19310	96825 ± 21601

**Discussion:** A High Dose subgroup was titrated upward to 300 mg/kg/day after 13 weeks dosing at 200 mg/kg/day, following Executive CAC recommendations (See minutes of ExecCAC meeting of December 9<sup>th</sup>, 2003, Fax to the Sponsor of December 11, 2003), due to anticipated development of exposure development and tolerance. However, excessive mortality was observed (See IND 61,345 SN 004, August 18, 2004) in the 300 mg/kg/day group, and the dose was reduced to 200 mg/kg/day, following Agency recommendations (see Memo to File IND 61,345, SN109, January 20, 2006). The following mortality was observed in Weeks 1-19 (table provided from the original NDA submission):

Group	1	2	3	4	5	
	Controls	50 mg/kg/day	100 mg/kg/day	200 mg/kg/day	200 mg/kg/day	300 mg/kg/day
Weeks	1-19	1-19	1-19	1-19	1-13	14-19
Males	0	0	1	0	7	5
Females	3	0	1	3	1	12

**Study title:** CG5503: 104-Week Oncogenicity (Feeding) Study in the Rat

**Key study findings:**

- Tapentadol can be considered negative for carcinogenic potential by daily oral (dietary) administration for 104 weeks at doses of up to 250 mg/kg/day in Wistar rats, under the conditions of this study
- Decreased body weights and body weight gains in the HD males (15% and -19%, respectively) and females (-15% and -18%, respectively) compared to negative controls at the end of the study
- Treatment-related hepatocellular hypertrophy in male and female rats at 125 and 250 mg/kg/day
- Agency statistical analyses detected positive trends in the incidence in female rats in liver hepatocellular adenoma ( $p < 0.025$ , incidence 0/100, 0/50, 0/50, 1/50 and 2/50 at 0, 10, 50, 125, and 250 mg/kg/day, respectively) with incidence of 2% and 4% at the mid- and high dose.
- Historical control incidence for the laboratory in female rats for hepatocellular adenoma is 2.74% (range 0.005-10.20%), and for thymic lymphoma is 0.29% (range 0.00-8.51%)
- There was a statistically significant dose response for increased liver adenomas + carcinomas in the female rats, but no statistically significant increases over controls in pairwise comparisons.
- The NOAEL and highest dose evaluated, 250 mg/kg/day ( $AUC_{0-24} = 328$  ng.h/ml in males and 1349 ng.h/ml in females), represents exposure of approximately 0.7 times in the males and 2.7 times in females the clinical exposure to the parent drug at the proposed MRHD of 600 mg/day (clinical  $AUC \approx 500$  ng.h/ml).
- Exposure to the metabolite tapentadol O-glucuronide at the highest dose tested represented approximately 27 times the exposure to the metabolite at the clinical MRHD, on an AUC basis.

Adequacy of the carcinogenicity study and appropriateness of the test model: The 2-year carcinogenicity study on tapentadol in SPF Wistar rats was conducted under GLP, received proper quality assurance inspections, and used an appropriate test model with adequate number of animals evaluated per dose. However, the animal caging, in groups of five instead of singly in this dietary study, was substandard, and may have contributed to the high variability in tapentadol exposure observed in the toxicokinetic analyses. The Standard parameters were measured, at appropriate time intervals. The oral (*ad libitum* dietary) route of administration was used in agreement with the proposed indication for oral clinical treatment, and the vehicle (commercial standard, pelleted rat maintenance diet) was appropriate. The doses were supported by previous 13-week dose selection study results, and received concurrence by the ExecCAC. Appropriate dual negative control groups were used. Survival was sufficient, with historically normal mortality incidences for this species and strain, to detect potential pre-, non-, and neoplastic changes in the histopathology examinations following 104 weeks of treatment. All animals including the premature sacrifices and rats found dead, with the exception of 1 MDM and 1 HDF, were examined microscopically. No peer histopathology examination

was conducted. For comparisons in this review, historical control values are presented. Toxicokinetic evaluation of systemic exposure to the parent drug and main glucuronide metabolite was conducted in an appropriate number of satellite animals, and the results demonstrated adequate exposure to both agents were submitted with the study report.

Evaluation of tumor findings: There were no treatment-related increases in neoplastic lesions observed at any dose level compared to controls values and exceeding historical control range for the laboratory. There was a trend toward increased hepatocellular adenomas (2/49 compared to 0/50 in each of the female control groups) and uterine adenomas (2/49 compared to 0/50 in one and 1/50 in the other female control group) were observed in the HDF (incidence  $\approx$  4% each in the HDF compared to 1.82% in the controls). Also, there was one additional hepatocellular carcinoma in the HDM (2/50) compared to control males (1/50 in each control group). There was a statistically significant dose response for increased liver adenomas + carcinomas in the female rats, but no statistically significant increases over controls in pairwise comparisons. Agency statistical analyses also detected positive trends in the incidence in female rats in liver hepatocellular adenoma ( $p < 0.025$ , incidence 0/100, 0/50, 0/50, 1/50 and 2/50 at 0, 10, 50, 125, and 250 mg/kg/day, respectively). Historical control data suggested that the tumor incidences are within the background for the strain in this laboratory.

There was an increased incidence of centrilobular hepatocellular hypertrophy in the high mid-dose (125 mg/kg/d) and high-dose (250 mg/kg/d) males and females. Also, increased thyroid follicular cell hypertrophy and focal follicular hyperplasia were observed in the high-dose females, compared to controls. In agreement with the Sponsor, the treatment-related non-neoplastic findings in the liver and thyroid are probably secondary to adaptive responses associated with treatment-related increased hepatic metabolic enzyme activities.

In conclusion, tapentadol can be considered to be negative for carcinogenicity in Wistar rats, under the conditions of this study. The highest dose tested represented approximately 0.7 times in the males and 2.7 times in the females the clinical exposure to the parent drug and approximately 27 times the exposure to the glucuronide metabolite in the males and females combined, at the MRHD of 600 mg/day tapentadol, on an AUC basis.

**Study no.:** TP2418

**Conducting laboratory and location:**

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**b(4)**

**Date of study initiation:** April 30, 2002

**GLP compliance:** Yes

**QA report:** yes (x) no ( )

**Drug CG5503 (tapentadol), lot # (Batch #s) CEWS 140, CEWS 113, CEWS 143, CEWS 112, CEWS 155, and E0001/100, and % purity: 97.7%, 97.7%, 97.5%, 97.9%, 97.6%, and 101.1%, respectively**

**CAC concurrence: Yes**

**Methods**

**Doses:** 0 (dual control groups received identical feed without test article), 10, 50, 125, and 250 mg/kg/day hydrochloride salt form

**Basis of dose selection:** Maximum tolerated dose (MTD); the results of a preliminary study in male and female Wistar rats at doses of 250-1000 mg/kg/day PO for 13 weeks showed decreased body weights (BW) in the males and reduced body weight gains (BWG) in males at all doses and in the females at 500 and 1000 mg/kg/day. Target organ toxicity was observed in the liver, indicated by hepatocellular hypertrophy in the males and females and increased gamma-glutamyltransferase (G-GT) in the males at 500 and 1000 mg/kg/day. The MTD level for the 2-year carcinogenicity study was based on slightly greater than 10% decrease in BWG in the males at 250 mg/kg/day in the absence of target organ toxicity, and the incidence of hepatocellular hypertrophy at 500 mg/kg/day in the females. The doses used in the pivotal carcinogenicity study received concurrence from the ExecCAC (meeting of January 22, 2002).

**Species/strain:** Wistar (SPF) rats

**Number/sex/group (main study):** 50/sex/group; the group assignments are presented in the following table (provided from the original NDA submission):

Allocation	Group 1*	Group 2*	Group 3	Group 4	Group 5	Group 6
And Target	0	0	10	50	125	250
Dose Levels	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
Males A	1-50	61-110	121-170	181-230	241-290	301-350
Males B	51-60	111-120	171-180	231-240	291-300	351-360
Females A	361-410	421-470	481-530	541-590	601-650	661-710
Females B	411-420	471-480	531-540	591-600	651-660	711-720

A = Oncogenicity Animals

B = Plasma Level Animals

\* Control animals received pelleted control diet without the test item

**Route, formulation, volume:** Test article mixed into pelleted standard rat maintenance diet, administered by feeding *ad libitum*

**Frequency of dosing:** *Ad libitum* in feed

**Satellite groups used for toxicokinetics or special groups:** 10/sex/group TK

**Age:** 4 weeks at initiation of dosing

**Weights:** 60.9-88.6 g males and 54.0-82.3 g females at initiation of dosing

**Animal housing:** The rats were housed 5/cage type-4 with sterilized standard softwood bedding) in an AAALAC-approved laboratory under Swiss Animal Protection Law (license No. 41); temperature 22 ± 3 deg.C, relative humidity 30%-70%, 10-15 air changes/hour, 12 hour light/dark cycle

b(4)

b(4)

**Restriction paradigm for dietary restriction studies:** None; Standard pelleted rat maintenance diet and water provided *ad libitum* (feed batches analyzed to confirm contaminant-free)

**Drug stability/homogeneity:** Content and homogeneity analyzed at first feed and at 3-month intervals; achieved doses were 99.5%-100.6% target doses during the dosing period; no test article found in the control feed batches; test article admixed with the feed weekly up to Week 13, and every 2 weeks from Week 14 to end of dosing period

**Dual controls employed:** Yes

**Interim sacrifices:** None

**Deviations from original study protocol:** No tissues available for histopathology examination from Animals 248 (125 mg/kg/d M) and 705 (250 mg/kg/d F) due to cannibalism following spontaneous deaths.

#### **Observation times**

**Mortality:** Twice daily

**Clinical signs:** Once daily

**Body weights:** Weekly to Week 13, then every 2 weeks until end of 104-week dosing period

**Food consumption:** Weekly to Week 13, then every 2 weeks until end of 104-week dosing period

**Hematology:** Non-fasted rats (allocation A animals) received blood draws from retro-orbital plexus at end of treatment (Week 104)

**Gross necropsy:** All moribund and surviving (104 weeks treatment) animals were examined macroscopically for abnormalities

**Histopathology: Peer review: yes ( ), no ( x ): The following tissues from all rats that died spontaneously, were terminated *in extremis*, and that survived to the end of the study were embedded, cut to 2-4 micrometers, stained with haematoxylin and eosin, and examined from all moribund and surviving (104 weeks treatment) animals: adrenal glands, aorta, auricles, bone (sternum and femur including joint), bone marrow, brain (medulla/pons, cerebral and cerebellar cortex), cecum, colon, duodenum, epididymides (fixed in Bouin's solution), esophagus, eyes with optic nerve (fixed in Davidson's solution), Harderian gland (fixed in Davidson's solution), head, heart, ileum, jejunum, kidneys, larynx, lacrimal gland (exorbital), liver, lungs (formalin filled at necropsy), lymph nodes (mesenteric, mandibular), mammary glands, nasal cavity, ovaries, pancreas, pharynx, pituitary gland, prostate gland, rectum, salivary glands (mandibular, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, mid-thoracic, lumbar), spleen, stomach, testes (fixed in Bouin's solution), thymus, thyroid including parathyroid gland, tongue, trachea, urinary bladder (formalin filled at necropsy), uterus with cervix, vagina, Zymbal's gland, and gross lesions and tissue masses**

**Toxicokinetics:** Blood samples (0.6-0.7 ml) collected (Allocation B animals) from the retro-orbital plexus in Weeks 4, 13, and 26

#### **Results**

**Mortality:** Survival was adequate at the end of the dosing period for valid statistical evaluation of the parameters examined. The deaths during the study (found dead and

sacrificed *in extremis*) and survival (percent) at the end of the dosing period are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	0 (Control)		0 (Control)		10		50		125		250	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Sex												
Number of animals												
At start	50	50	50	50	50	50	50	50	50	50	50	50
Died or sacrificed moribund	8	15	14	15	17	14	16	13	15	13	12	11
Terminal sacrifice	42	35	36	35	33	36	34	37	35	37	38	39
Survival (%)	84	70	72	70	66	72	68	74	70	74	76	78

**Clinical signs:** No treatment-related effects

**Body weights:** Statistically significant reduction in BW in HD (250 mg/kg/d) males from week 16 onward and HD females from week 7 onward to the end of the dosing period. Statistically significant reduction in BWG in the HD males from week 11 onward and HD females from week 34 onward to the end of the dosing period.

The results of the body weight and body weight gain observations at the end of the study are presented in the following table (provided from the original NDA submission, percent change from Group 1 controls):

Week 104	Group (mg/kg/d)						
	Sex	1 (0)	2 (0)	3 (LD) (10)	4 (MD1) (50)	5 (MD2) (125)	6 (HD) (250)
Body Weight (% difference from controls)	M	Reference	-4.2%	+4.1%	-0.7%	-1.9%**	-14.8%**
	F	Reference	+3.8%	+1.6%	-6.9%	-7.6%**	-15.4%**
Body Weight Gain (% difference from controls)	M	Reference	-5.8%	+1.3%	+0.9%	-4.9%	-19%**
	F	Reference	+6.9%	+4.6%	-7.4%	-8.9%	-18%**

\*\*significant at p<0.01

**Food consumption:** No treatment-related effects in the males; HD (250 mg/kg/day) F showed increased mean absolute food consumption from Weeks 24-38 compared to controls. At the end of the study, food consumption was increased 20.0% in the males and females at the HD (statistically significant at p<0.01). The increase in food consumption may have resulted in higher doses than reported, in these animals.

**Hematology:** No treatment-related effects

**Gross pathology:** No treatment-related effects, including nodules and masses

**Histopathology:**

**Non-neoplastic:**

- Statistically significant increase compared to controls in centrilobular hepatocellular hypertrophy in the MD2 (125 mg/kg/d) M (incidence 24/49) and F (34/50), and in the HD (250 mg/kg/d) M (45/50) and F (43/49)

- Increased follicular cell hypertrophy and focal follicular hyperplasia in the HD F, when compared with the combined dual control group incidence, attributed to enhanced liver enzyme activities and hypertrophy, by the Sponsor
- The non-neoplastic hepatic effects are probably secondary to adaptive response associated with treatment-related liver enzyme activities, in agreement with the Sponsor

The noteworthy findings in the histopathology examination for non-neoplastic lesions are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	0 (Control)		0 (Control)		10		50		125		250	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Sex												
Histopathology – non-neoplastic lesions												
Liver – no. evaluated	50	50	50	50	50	50	50	49	49	50	50	49
Centrilobular hepatocellular hypertrophy	-	-	-	-	-	-	-	-	24**	34**	25**	43**
Thyroid	50	49	50	48	50	49	50	46	49	49	50	49
follicular cell hypertrophy	26	16	10	1	24	10	15	17	22	18	30	23**
focal cell hyperplasia	7	1	4	1	3	2	6	1	2	3	5	7**

\*\* significant at p < 0.005

**Neoplastic:** No statistically significant treatment-related effects, although Agency statistical analyses revealed positive trends in the incidence in female rats in liver hepatocellular adenoma (p<0.025, incidence 0/100, 0/50, 0/50, 1/50 and 2/50 at 0, 10, 50, 125, and 250 mg/kg/day, respectively). The results of the histopathology examination for neoplastic lesions are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	Number of animals with neoplastic lesions:											
	0 (Control)		0 (Control)		10		50		125		250	
Sex	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Hemolymphoret. system												
no. evaluated	2	1	3	1	-	2	4	3	-	1	-	-
malignant lymphoma	1	-	3	1	-	2	4	2	-	1	-	-
histiocytic sarcoma	1	1	1	-	-	-	-	1	-	-	-	-
Heart – no. evaluated	49	50	50	49	50	50	50	47	49	48	50	49
malignant atrio caval mesothelioma	-	-	-	-	-	-	-	-	-	1	-	-
metastasis of carcinoma	-	-	1	-	-	-	-	-	-	1	-	-
Liver – no. evaluated	50	50	50	50	50	50	50	49	49	50	50	49
hemangioma	-	1	-	-	-	-	-	-	-	-	-	-
hepatocellular adenoma	-	-	-	-	-	-	-	-	-	1	-	2
hepatocellular carcinoma	1	-	1	-	-	-	-	-	1	1	2	-
metastasis fo carcinoma	-	-	-	-	-	-	-	1	-	2	-	-
Spleen – no. evaluated	50	49	50	50	50	50	50	49	49	49	50	49
leiomyoma	1	-	-	-	1	-	-	-	-	-	-	-
hemangio sarcoma	-	-	-	-	-	-	1	-	-	-	-	-
contact metastasis	-	-	1	-	-	-	-	-	-	-	-	-
metastasis of carcinoma	-	-	-	-	-	-	-	1	-	-	-	-

Daily dose (mg/kg)	0 (Control)		0 (Control)		10		50		125		250	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Sex												
Mesenteric lymph node – no. evaluated	50	49	50	50	48	49	49	49	49	48	50	49
hemangioma	2	1	1	-	1	-	1	1	3	1	-	-
hemangiosarcoma	-	-	-	-	-	-	1	-	-	-	-	-
metastasis of carcinoma	-	-	1	-	-	-	-	-	-	1	-	-
Kidneys – no. evaluated	49	50	50	50	50	50	50	49	49	49	50	49
renal lipoma	1	-	-	-	-	-	-	-	-	1	-	-
tubular cell adenoma	1	-	-	-	2	-	1	1	1	-	-	1
tubular cell carcinoma	-	-	-	-	1	-	1	1	-	-	-	1
mesenchymal tumor	-	-	1	-	1	-	-	-	1	-	-	-
metastasis of carcinoma	-	-	-	-	-	-	-	-	-	1	-	-
Stomach – no. evaluated	49	49	50	50	50	5	50	48	49	48	50	49
contact metastasis	-	-	1	-	-	-	1	-	-	1	1	-
leiomyosarcoma	1	-	-	-	-	-	-	-	-	-	-	-
Lung – no. evaluated	49	49	50	49	50	49	50	48	49	48	50	49
metastasis of carcinoma	-	-	1	-	-	-	1	-	-	1	1	-
metastasis of sarcoma	1	-	-	1	-	-	-	-	-	-	-	-
Daily dose (mg/kg)	0 (Control)		0 (Control)		10		50		125		250	
Sex	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Thymus – no. evaluated	49	49	50	48	49	48	49	47	48	48	50	49
benign thymoma	1	-	-	2	1	5	-	2	-	2	-	2
thymic lymphoma	2	1	-	-	1	-	1	1	1	3	-	1
metastasis of carcinoma	-	-	-	-	-	-	-	-	-	1	-	1
Testes – no. evaluated	49	-	50	-	50	-	50	-	49	-	50	-
benign Leydig cell tumor	1	-	1	-	1	-	-	-	-	-	-	-
benign mesothelioma	-	-	1	-	-	-	-	-	-	-	-	-
Ovaries – no. evaluated	-	49	-	48	-	48	-	47	-	48	-	49
sex cord stromal tumor	-	-	-	-	2	-	1	-	2	-	1	-
benign thecoma	-	-	-	-	1	-	-	-	1	-	-	-
benign granulosa cell tumor	-	1	-	-	-	-	-	-	-	-	-	1
contact metastasis	-	-	-	-	-	-	1	-	1	-	-	-
Epididymids – no. evaluated	49	-	50	-	50	-	50	-	49	-	50	-
benign mesothelioma	-	-	1	-	-	-	-	-	-	-	-	-
Pancreas – no. evaluated	47	50	49	49	48	49	48	48	48	48	50	49
islet cell adenoma	-	-	2	-	3	-	2	-	-	3	1	-
mixed acinar cell adenoma	-	-	-	-	-	-	-	1	1	-	-	-
contact metastasis	-	-	1	-	-	-	-	-	-	1	-	-
island cell carcinoma	-	1	-	-	-	-	-	-	-	1	-	-
Daily dose (mg/kg)	0 (Control)		0 (Control)		10		50		125		250	
Sex	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Thyroid gland – no. evaluated	50	49	50	48	50	49	50	46	49	49	50	49
C-cell adenoma	3	5	2	2	5	5	2	6	4	4	4	7
C-cell carcinoma	-	-	-	1	-	-	-	-	-	1	-	-
follicular cell adenoma	2	1	1	-	-	2	-	2	2	1	3	-
follicular cell carcinoma	-	-	-	-	-	-	-	1	-	-	1	-
Adrenal cortices – no. evaluated	49	50	50	50	50	50	50	49	49	49	50	49
adenoma	1	-	-	-	-	-	-	-	1	-	-	-
adenocarcinoma	-	-	-	-	-	-	-	-	-	-	1	-
metastasis of carcinoma	-	-	-	-	-	-	-	-	-	2	-	-
Adrenal medullas – no. evaluated	48	50	50	50	50	50	50	49	49	49	50	49
benign pheochromocytoma	2	1	1	1	1	-	-	2	-	-	3	-
malignant pheochromocytoma	-	-	-	-	1	-	1	-	-	1	-	-
Skin non-routine – no. evaluated	8	1	8	7	8	6	10	5	6	4	7	3
fibrosarcoma	-	1	1	2	-	-	-	-	-	-	-	-
keratoacanthoma	1	-	3	1	2	-	4	-	2	-	1	-
osteosarcoma	1	-	-	-	-	-	-	-	-	-	-	-
lipoma	1	-	-	-	-	-	2	1	-	-	-	-
fibroma	-	-	1	-	2	-	2	-	-	1	1	-
squamous cell carcinoma	-	-	-	-	1	1	-	1	-	-	-	-
basal cell carcinoma	-	-	-	-	-	-	-	-	-	1	-	-
sebaceous cell adenoma	-	-	-	-	-	-	1	-	-	-	-	-
malignant schwannoma	-	-	-	-	-	1	-	-	-	-	-	-

Daily dose (mg/kg)	0 (Control)		0 (Control)		10		50		125		250	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Sex	50	50	50	49	50	50	50	49	49	50	50	49
Cerebrum - no. evaluated	-	-	-	-	-	-	-	-	-	-	1	1
meningioma	-	-	-	-	-	-	-	-	-	-	-	-
granular cell tumor	-	-	-	-	-	-	1	-	-	-	1	-
ependynoma	-	-	-	1	-	1	-	-	-	-	-	-
Cerebellum - no. evaluated	50	50	50	49	50	50	50	49	49	50	50	49
benign astrocytoma	-	-	1	-	-	-	-	-	-	-	-	-
menangioma	-	-	-	-	-	-	-	-	-	-	1	-
Seminal vesicles - no. evaluated	50	50	50	49	50	50	50	49	49	50	50	49
leiomyosarcoma	-	-	-	-	-	-	-	1	-	-	-	-
contact metastasis	-	-	1	-	-	-	-	-	-	-	-	-
Pituitary gland - no. evaluated	50	50	50	49	50	50	50	48	48	50	50	49
ganglioneuroma (pars nervosa)	-	-	-	-	-	-	1	-	-	-	1	-
adenoma of pars distalis	13	16	16	19	19	20	20	23	23	16	16	19
Mammary gland	50	50	50	50	50	50	50	50	50	50	50	49
mammary fibrosa	2	3	3	3	1	1	1	4	4	4	1	1
fibroadenoma	11	9	9	8	8	8	7	7	7	9	9	9
adenoma	1	1	1	3	3	1	4	4	4	2	2	2
adenocarcinoma	3	1	1	2	2	7	1	1	1	3	3	3
Clitoral gland	-	-	-	-	-	-	1	-	-	-	-	-
adenoma	-	-	-	-	-	-	1	-	-	-	-	-

Daily dose (mg/kg)	0 (Control)		0 (Control)		10		50		125		250	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Sex	50	50	50	49	50	50	50	49	49	50	50	49
Uterus - no. examined	1	-	-	-	-	-	-	-	-	-	-	1
glandular polyp	6	7	7	3	4	2	2	2	2	9	9	9
endometrial-stromal polyp	-	-	-	-	-	-	1	-	-	-	-	1
granular cell tumor	-	-	-	-	-	-	-	1	-	-	-	-
squamous cell tumor	-	-	-	-	-	-	-	-	1	-	-	-
hemangioma	1	-	-	-	-	-	-	-	1	-	-	-
adenoma	-	1	-	-	-	-	-	-	-	-	2	-
leioma	-	-	-	-	-	-	1	-	-	-	-	-
leiomyosarcoma	1	-	-	-	-	-	-	-	-	-	-	1
adenocarcinoma	1	-	-	-	-	2	1	1	1	2	2	2
endometrial-stromal sarcoma	-	1	1	1	-	-	-	1	1	-	-	-
contact metastasis	-	-	-	-	-	-	-	-	1	-	-	-
Cervix - no. examined	50	49	49	49	49	48	48	49	49	49	49	49
fibroma	-	-	-	-	-	-	-	-	-	-	-	-
leiomyoma	-	-	-	-	-	-	-	-	-	-	-	-
granular cell tumor	-	-	-	-	-	-	-	-	-	-	-	-
contact metastasis	-	-	-	-	-	-	-	-	-	-	-	-
stromal cell carcinoma	-	-	-	-	-	-	-	-	-	-	-	-

Daily dose (mg/kg)	0 (Control)		0 (Control)		10		50		125		250	
	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
Sex	50	50	50	49	50	50	50	48	49	49	50	49
Vagina - no. examined	-	-	-	-	-	1	-	-	-	-	-	-
leiomyoma	-	1	1	-	-	-	-	-	-	-	-	-
contact metastasis	-	-	-	-	-	-	-	-	-	-	-	1
metastasis of sarcoma	-	-	1	-	-	-	-	-	-	-	-	-
adenoma polyp	-	-	-	-	-	-	-	-	-	-	-	-
granular cell tumor	-	-	-	-	-	-	1	-	-	-	-	-
Iliac lymph node - no. evaluated	-	-	1	-	-	-	-	-	-	-	1	-
hemangioma	-	-	1	-	-	-	-	-	-	-	-	-
Cervical lymph node - no. evaluated	1	-	-	-	-	-	-	-	-	-	-	-
metastasis of sarcoma	1	-	-	-	-	-	-	-	-	-	-	-
Paranasal sinuses	-	-	1	-	-	-	-	-	-	-	-	-
benign schwannoma	-	-	1	-	-	-	-	-	-	-	-	-

Historical control data for the notable neoplastic findings are presented below for comparison (provided from the original NDA submission):